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# EPIDEMIC POLIOMYELITIS

## Some Pathologic Observations on Human Material

JOSEPH A. LUHAN, M.D., Ph.D.

CHICAGO

THE BASIS of this study was a series of 13 fatal cases of poliomyelitis coming to necropsy at the Cook County Hospital during the epidemic of poliomyelitis that occurred in Chicago and Cook County in 1943. During this period permission to make a necropsy was obtained in 15 cases diagnosed as cases of poliomyelitis, but the first of these proved to be one of toxic encephalopathy associated with pneumonia, and another, meningococcic septicemia with very early or minimal cerebral leptomeningitis.

During 1943 there were 1,262 cases of poliomyelitis reported in Cook County, with 109 deaths, the case fatality rate being almost 8.6 per cent.[1]

### CLINICAL FEATURES OF THE DISEASE (SEE TABLE 1)

The patients in this series ranged in age from 4 to 34 years, with a median age of 8 years.

The duration of the illness varied from three to twenty-one days, with a median duration of six days. However, death occurred from two hours to twelve days (median, nineteen hours) after admission to the hospital.

The neurologic examinations of most of these desperately ill patients were superficial, so that it was not possible to correlate carefully the topography of the histologic lesions with the localization of the paralysis. Clinically, the patients in this series all showed "bulbar" involvement, but this was recorded as severe on admission in only 7 cases. Seven patients were observed to have no obvious paralysis of the extremities on admission to the hospital. Respiratory failure predominantly of the spinal type with intercostal paralysis was observed in 3 subjects. Four patients were treated in a Drinker respirator. Three showed terminal hyperthermia. Cerebrospinal fluid obtained by spinal puncture in 6 cases revealed cell contents ranging from 100 to 480 cells per cubic millimeter.

This study was aided by a grant from the National Foundation for Infantile Paralysis.

From the Department of Neurology and Psychiatry, Loyola University School of Medicine, and the Neuropathological Laboratory of the Cook County Psychopathic Hospital.

1. Vital Statistics Bulletin, Yearly Report, Illinois Department of Public Health, 1943.

TABLE 1.—*Summary of the Clinical Features in This Series of Cases of Poliomyelitis*

| Case and Patient's Initials | Age (Yr.), Sex and Color | Sequential Mode of Onset | Total Duration of Illness | Duration After Admission to Hospital | Clinical Severity of Bulbar Symptoms | Clinical Evidence of Spinal Paralysis on Admission | Comment on Clinical Course |
|---|---|---|---|---|---|---|---|
| 1. W. K. | 10 M White | Fever, listlessness, abdominal pain, then bulbar symptoms | 5½ days | 10 hours | 3+ | Areflexia; patient able to move extremities | Terminal hyperpyrexia (107.4 F., rectal) |
| 2. F. D. | 10 M White | Sore throat, fever, then difficulty in swallowing | 4 days | 25 hours | 3+ | Questionable left-sided foot drop; patient moved all extremities; deep reflexes 1+ | Patient became stuporous and had nystagmus; terminal hyperpyrexia (109.6 F., rectal) |
| 3. T. C. | 20 M White | Sore throat, vomiting, headache; bulbar symptoms 12 hours before admission | 5 days | 5½ hours | 2+ | No obvious paralysis; both knee jerks and right ankle jerk absent | |
| 4. J. R. | 6 M White | Vomiting, diarrhea, fever; remission 3 days; then difficulty in swallowing and fever (dromedary type) | 6¼ days | 6½ hours | 4+ | No apparent paralysis; deep reflexes all present | Patient stuporous, with body tremors; pupils dilated; involuntary movements of eyeballs |
| 5. E. D. | 18 F White | Headache, stiff neck and vomiting; next day severe vertigo | 6½ days | 4½ days | 3+ | None; deep reflexes present, abdominal reflexes absent | Intercostal paralysis and weakness of right arm developed later; patient became irrational; shortly before death temperature was 107 F. (rectal), pulse rate 155, respirations 40 |
| 6. B. K. | 8 F White | Difficulty in swallowing, temperature of 106 F. (oral), restlessness | 7 days | 6 hours | 3+ | Slight weakness of lower extremities | Nystagmus; circulatory collapse developed with marked cyanosis; terminal temperature, 105 F. (rectal) |
| 7. H. W. | 7 M White | Fatigue, restlessness, nasal speech; next day stiff neck, dysphagia | 6 days | 19 hours | 3+ | No apparent weakness of extremities | Patient in Drinker respirator for 5 hours before death |
| 8. E. E. | 6 M Negro | Headache and repeated vomiting for 3 days, abdominal pain a few hours before admission | 3 days | 2 hours | 1+ | No gross paralysis in extremities; deep reflexes (except biceps jerks) and abdominal reflexes absent | Temperature 103.8 F. (rectal), respirations 52, pulse rate 160 on admission; patient had marked difficulty in breathing |
| 9. W. J. | 5½ M White | Listlessness; next day sore throat and fever; remission for a few days, then stiff neck, fever, weakness of arms (dromedary type) | 9 days | 53 hours | 1+ | Marked weakness of both upper extremities although deep reflexes were still present | On second hospital day marked bulbar symptoms developed, and patient was placed in Drinker respirator but failed rapidly |
| 10. R. M. | 4 M White | Headache, fever and vomiting; next day difficulty in swallowing | 3 days | 23 hours | 3+ | No paralysis; all deep reflexes brisk, as were abdominal reflexes | Shortly before death rectal temperature 104 F., respirations 40, pulse rate 140 |
| 11. F. P. | 3¾ M White | No history of mode of onset available | 21 days | 12 days | 1+ | Slight generalized weakness of extremities, marked intercostal paralysis | Patient very dyspneic on admission and placed immediately in respirator; at one time was able to remain outside the respirator for 27 minutes |
| 12. R. D. | 9 F White | Fever (102 F.), headache; three days later vomiting and dyspnea | 6 days | 2 days | 2+ | Intercostal paralysis; no note of paralysis of limbs made | Patient, an achondroplastic dwarf, placed in respirator a few hours after admission |
| 13. F. W. | 34 F White | "Intestinal influenza," abdominal pain, diarrhea for two days; remission; on 7th day dyspnea, dysphagia, coma | 7 days | 4 hours | 2+ | Deep reflexes "diminished to absent," but patient was in deep coma | Patient died after 9 hours in deep coma; had had a convulsive seizure 2 hours after admission |

### GROSS PATHOLOGIC CHANGES

The extraneural pathologic changes included well marked swelling congestion or hyperplasia of lymph nodes of the intestine and the mesentery in 6 patients. Twelve patients showed varying degrees of pulmonary congestion and edema, but only 1 had frank pneumonia (bronchopneumonia of the lower lobe of the right lung). One patient presented verrucous mitral endocarditis, without any embolic features or evidence of chronic congestive decompensation.

The brain appeared edematous to a slight or a moderate degree in 12 cases. Hyperemia of the cord and the brain stem was regularly encountered, but in only 1 case (7) were petechial hemorrhages seen within the neuraxis, and these were encountered in the thoracic part of the spinal cord.

### HISTOLOGIC CONSIDERATIONS AND OBSERVATIONS

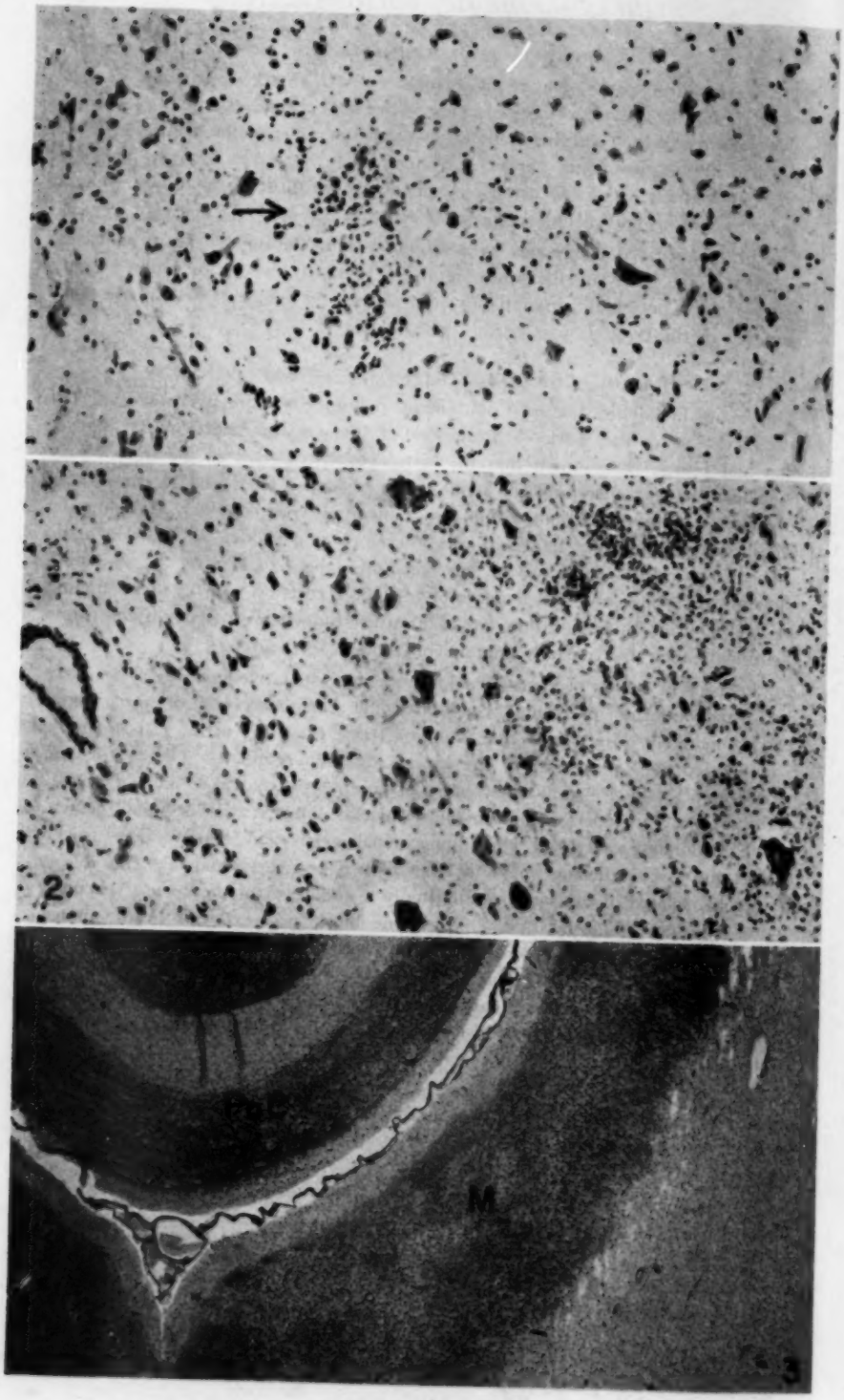
The main histologic features of human poliomyelitis are well known, but within recent years the work of a number of investigators, particularly that of Bodian and Howe,[2] has shown that the histologic distribution of the lesions may yield valuable clues concerning the mode of entry of the virus and its subsequent propagation and dissemination within the central nervous system. Examination of the present neurologic material was therefore undertaken with topographic relationships as one objective. Also, it seemed advisable to add a record of certain peculiarities of localization and nuances of histologic reaction seen in this sampling of the 1943 Chicago epidemic to the ample literature for whatever it might contribute to identification of the virus on anatomicopathologic grounds.

In the present series of cases, blocks from representative cortical areas were studied: the anterior prefrontal convexity, the basilar region of the frontal cortex immediately adjacent to the olfactory tract, premotor areas (Brodman 6), the motor cortex, particularly the area gigantopyramidalis (FA$\gamma$ of von Economo and Koskinas) near the vertex or in the paracentral lobule; the postcentral cortex; the superior and inferior parietal lobules; the occipital cortex, including Brodmann's area 17; the hippocampal gyrus and region of the cornu ammonis; the gyri bordering on the sylvian fissure, and the island of Reil. These were merely isolated blocks, and so admittedly a good deal of the cortex remained unstudied; moreover, in the first 3 cases, one block was taken from the motor area of one side only (in addition to other cortical blocks) and the remainder of the brain discarded before this study was contemplated. Cresyl violet or toluidine blue, hematoxylin and eosin, and Weil stains were routinely used on sections cut from material embedded in paraffin and pyroxlin (nitrocellulose) (soluble cotton [20 to 25 per cent water]).

There were only 5 cases (3, 4, 5 8 and 10) in which fairly complete olfactory bulbs and tracts were received for examination. Partial serial sections of these bulbs and tracts showed normal appearances. In all of these cases the medulla oblongata was severely involved.

In 12 of the 13 cases the gigantocellular motor area was involved by some degree of interstitial cellular infiltration with or without unmistakable perivascular cuffing. In the remaining case (3) only one small paraffin-embedded block was available from the left motor area; this appeared normal except for a few subcortical perivenous accumulations of mononuclears and scavenger cells.

2. Howe, H. A., and Bodian, D.: Neural Mechanism in Poliomyelitis, New York, Commonwealth Fund, 1942.




FIGURES 1 TO 3

*(See legends on opposite page)*

It is possible that if more blocks through the motor area of this brain had been studied, typical cortical infiltrative changes might have been found.

In severity these cortical infiltrates were rated as minimal in 5 cases, mild in 4, and prominent and unmistakable in the remaining 3 (figs. 1 and 2). I should venture to guess that if a quick survey of the sections of the motor area were made as in routine histologic study one could easily miss the insignificant and scattered involvement of the motor area in about half of the cases. In 3 instances of minimal involvement only serial sectioning of the block revealed the changes, although in most cases the first section of the gigantocellular area encountered disclosed the inflammatory reaction. The disappearance of interstitial infiltration as soon as an adjacent cytoarchitectural area was reached was striking (fig. 3).

Interstitial cortical infiltrations were found only in the gigantocellular area except in 1 case (9), and that presented the most pronounced involvement of the motor area in the whole series. The site of these other foci of infiltration of cortical tissue was in the hippocampal region, where they occurred in the form of occasional tiny cellular foci and borderline perivascular infiltrations in the upper cortical layers, underlying slight mononuclear infiltration of the leptomeninges, not far removed from moderately severe poliomyelitic involvement of the substantia nigra at this level.

The interstitial infiltrates in the motor cortex were often associated with mild mononuclear infiltration of the regional leptomeninges and small subcortical perivascular mononuclear and scavenger cell infiltrations. However, the leptomeningeal reaction was not limited to the motor area; it was seen particularly well developed in 1 case (4) in which mild discontinuous mononuclear infiltrations were found in the hyperemic and edematous leptomeninges over the frontal, temporal and parietal cortex.

Slight perivascular infiltration was noted in other cortical regions in addition to the motor area in 3 cases; in 2 instances the hippocampal region was affected, and in a third, the premotor cortex.

The intraparenchymal cellular infiltrates in the motor cortex were often predominantly microglial in nature (fig. 4) except in a few cases where polymorphonuclear cells morphologically typical of leukocytes were conspicuous, presumably indicative of an early process (fig. 5). In other instances a mixed polymorphonuclear and rod cell infiltrate was found (fig. 6). The infiltrations were both focal and diffuse and were situated for the most part within the third to the fifth cortical layers. Occasional neuronophagic pictures were encountered, proportional to the severity of the interstitial infiltrate. These sometimes involved Betz cells (fig. 7) but more often implicated small ganglion cells within the motor area. Sometimes the ganglion cells showed morphologic abnormalities without microgliosis or cellular infiltration of their immediate neighborhood (fig. 8).

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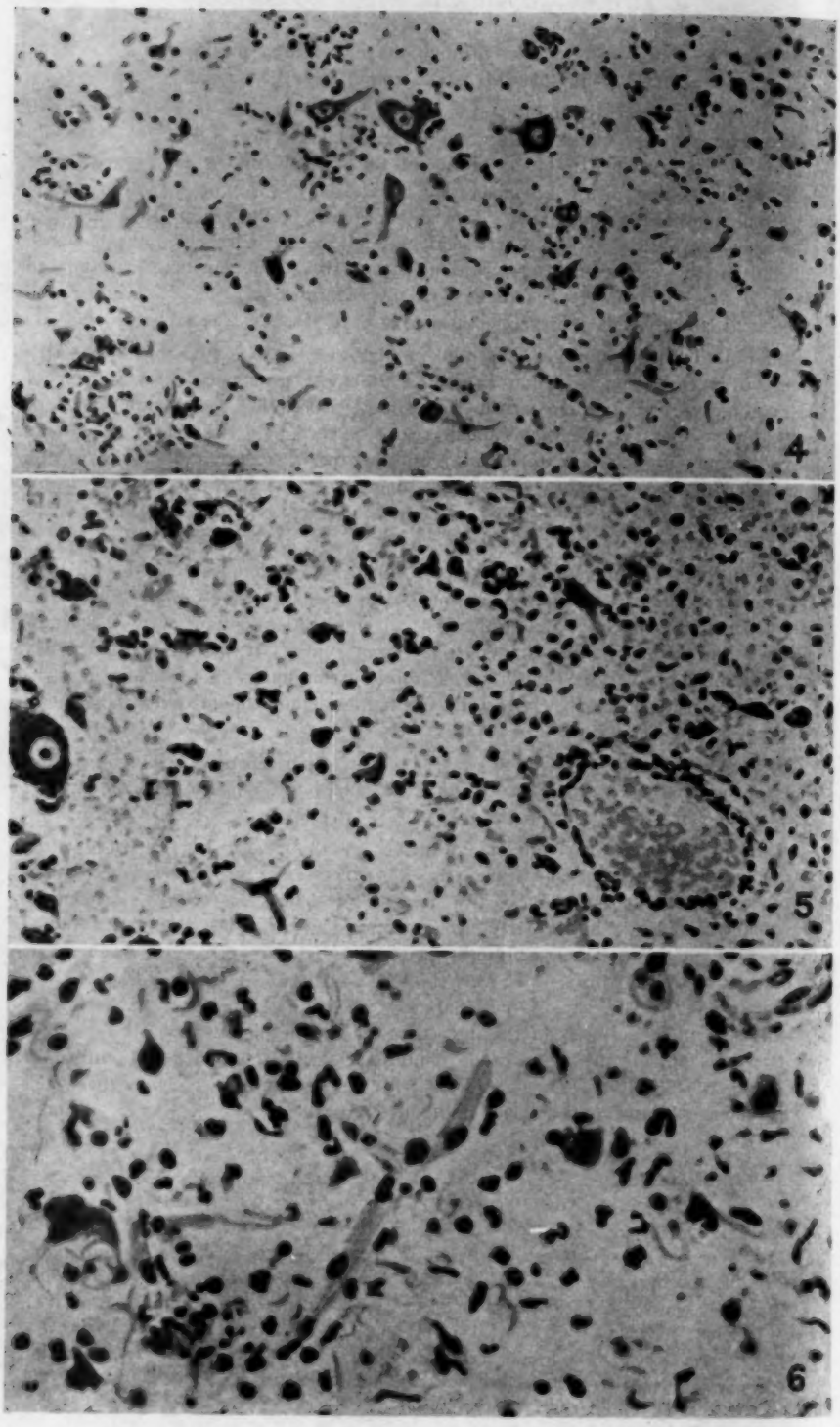
EXPLANATION OF FIGURES 1 TO 3

Fig. 1 (case 1).—A typical minimal intraparenchymal infiltrate in the motor area of the cerebral cortex. Cresyl violet; × 150.

Fig. 2 (case 9).—Marked poliomyelitic involvement of the right motor area. Cresyl violet; × 120.

Fig. 3 (case 9).—Photomicrograph showing the poliomyelitic inflammation sharply limited to the motor area (*M*). The adjacent posterior central gyrus (*PoC*) is uninvolved. Cresyl violet; × 14.




FIGURES 4 TO 6

*(See legends on opposite page)*

Most estimates of the comparative severity of inflammatory reactions within different regions of the neuraxis are relatively crude unless the tremendous task of counting cells and abnormal formations in serial section is undertaken as a basis for comparisons. One should distinguish in poliomyelitis between (1) perivascular infiltrations, (2) intraparenchymal cellular infiltrations and (3) histologic appearances of varying degrees of damage or destruction of ganglion cells.

Even when one disregards the relative degree and importance of these different components of inflammatory reaction and considers the severity of the reaction as a whole, one is still merely attempting to assay the intensity of pathologic change per area or volume of nervous tissue—which neither expresses the total volume-density of the inflammatory process nor assesses the biologic value of the part affected.

A quantitative appraisal of the total number of ganglion cells wiped out in the inflammatory process would probably disclose that the average cellular destruction for this present series among the given areas was highest in the cervical part of the spinal cord.

Considering the severity of all the parenchymatous manifestations as a whole (perivascular infiltration, infiltration of tissue and destruction of ganglion cells), one finds that the medulla oblongata was more severely involved than the cervical part of the spinal cord in 3 cases, was about as severely involved as that part of the cord in 6 instances and was less heavily implicated than the cervical part of the cord in 4 cases. Perivascular infiltration was usually more pronounced in the brain stem, particularly in the region underlying the lower part of the floor of the fourth ventricle, than in the cord. The severity of the parenchymatous process on the average diminished in a rostral direction from the medulla or the cervical part of the cord in most cases. The cord (especially the cervical part) and the brain stem, including the red nucleus and the substantia nigra, were more or less severely involved in most cases; the subthalamus and the hypothalamus were moderately implicated; the thalamus and the lenticular nuclei were relatively slightly involved (mainly by sparse perivascular infiltrations), and the cortex was usually unaffected except for slight discontinuous and selective implication of the motor area. The posterior part of the hypothalamus, as a rule, was more densely involved than the anterior part. Parenchymatous (in contrast to leptomeningeal) inflammation of the cerebellum was conspicuously lacking except within the roof nuclei and the dentate nuclei. The dentate nuclei, studied in 12 cases, showed interstitial lesions and destruction of ganglion cells in 11 cases and only perivascular infiltration in 1.

A crude sampling of the comparative total inflammatory intensity per area was attempted in the following manner: The usual 0 to 4 plus estimates of

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EXPLANATION OF FIGURES 4 TO 6

Fig. 4 (case 1).—High power view of a diffuse cellular infiltration, chiefly microglial, in the fifth layer of the motor cortex. Cresyl violet; × 180.

Fig. 5 (case 12).—Numerous polymorphonuclear leukocytes apparently streaming into the nervous tissue (motor cortex) from the small vessel. Cresyl violet; × 290.

Fig. 6 (case 9).—High power view of an infiltrate in the fifth layer of the motor cortex of the left hemisphere. Note the admixture of polymorphonuclear leukocytic and microglial (rod cell and polyblastic) forms in the infiltrate and the swelling of capillary endothelium. Cresyl violet; × 535.

the severity of the visible pathologic alterations were made (figs. 9 and 10) for a number of sections at various levels through a given region, ascribing the same weight to perivascular infiltration and infiltration of tissue as to cellular destruction, and these averaged for all the brain and cord specimens for each area. Since these were biased because of varying discontinuity of the disease process,

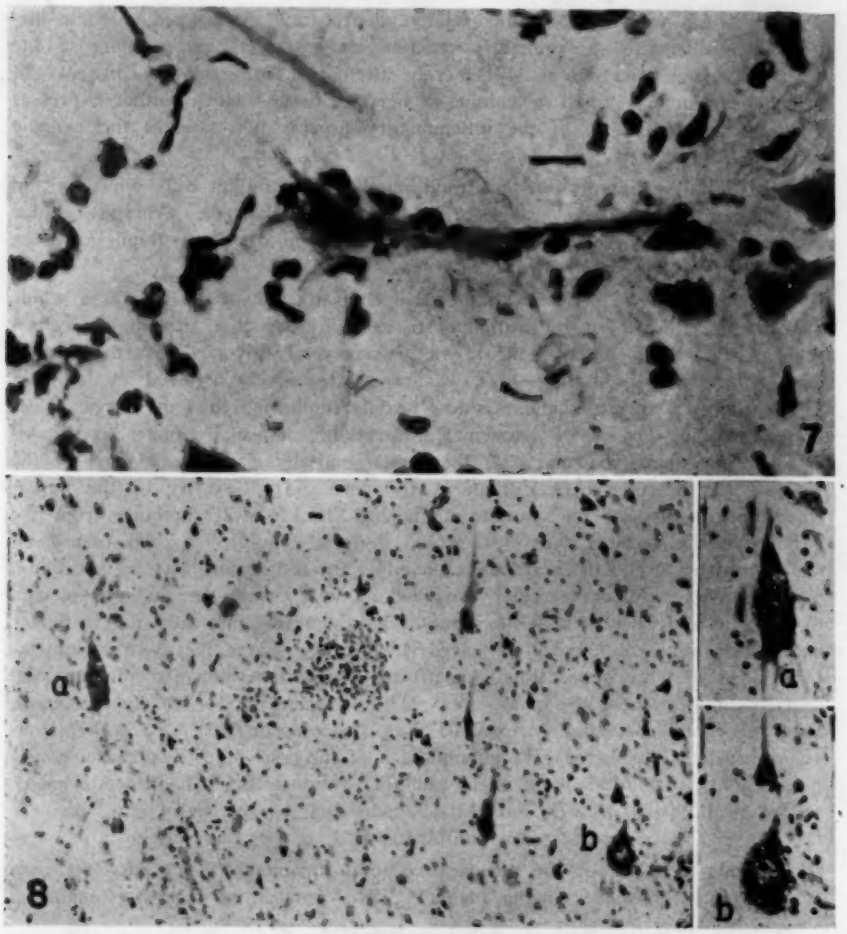



Fig. 7 (case 2).—Microglial proliferation and coffin formation neuronophagia of a Betz cell in the motor cortex. Cresyl violet; × 600.

Fig. 8 (case 12).—Focus of microgliosis as well as minimal diffuse cellular infiltration in the fifth layer of the motor cortex; the Betz cell marked *a* is severely damaged, in contrast with the normal-appearing cell *b* in the same field. Cresyl violet; × 125 and × 225.

a further adjustment was made for severity per total area (thus only a fraction of the total area of the motor cortex showed, say, 1 or 2 plus change), and the final numerical average estimates (from 0 to 4 plus) were multiplied by 10.

This gave the values for certain areas selected for comparison shown in table 2. Such numerical estimates connote much greater accuracy of observation than actually was possible. If visible neuronophagia and liquefaction necrosis had been selected as the criterion of severity, the cervical part of the cord would have a much higher rating than the rest of the neuraxis; but since it is probable that ganglion cells in the vicinity of a cellular exudate may be functionally damaged without showing gross staining abnormalities, the total density of cellular accumulation was used instead as the determining factor in the estimate. Where there is smoke, there is fire.

As previously mentioned, the densest perivascular infiltrations within the neuraxis in poliomyelitis tend to occur in the floor of the fourth ventricle (fig. 11). In the midbrain the red nuclei and the substantia nigra are usually the most severely involved (fig. 12). In the pons the locus ceruleus and the tegmental reticular formation generally are the most hard hit (fig. 13). Sometimes considerable involvement of one or both facial nuclei occurs, with neuronophagia (fig. 14). However, the basis pontis is almost unaffected in most instances, a circumstance difficult to explain if the virus travels along pyramidal pathways, unless the cells of the nuclei pontis are peculiarly immune to the virus. In the medulla the nuclei underlying the floor of the fourth ventricle, irrespective of

TABLE 2.—*Comparative Intensity of the Total Visible Pathologic Alterations in Certain Regions*

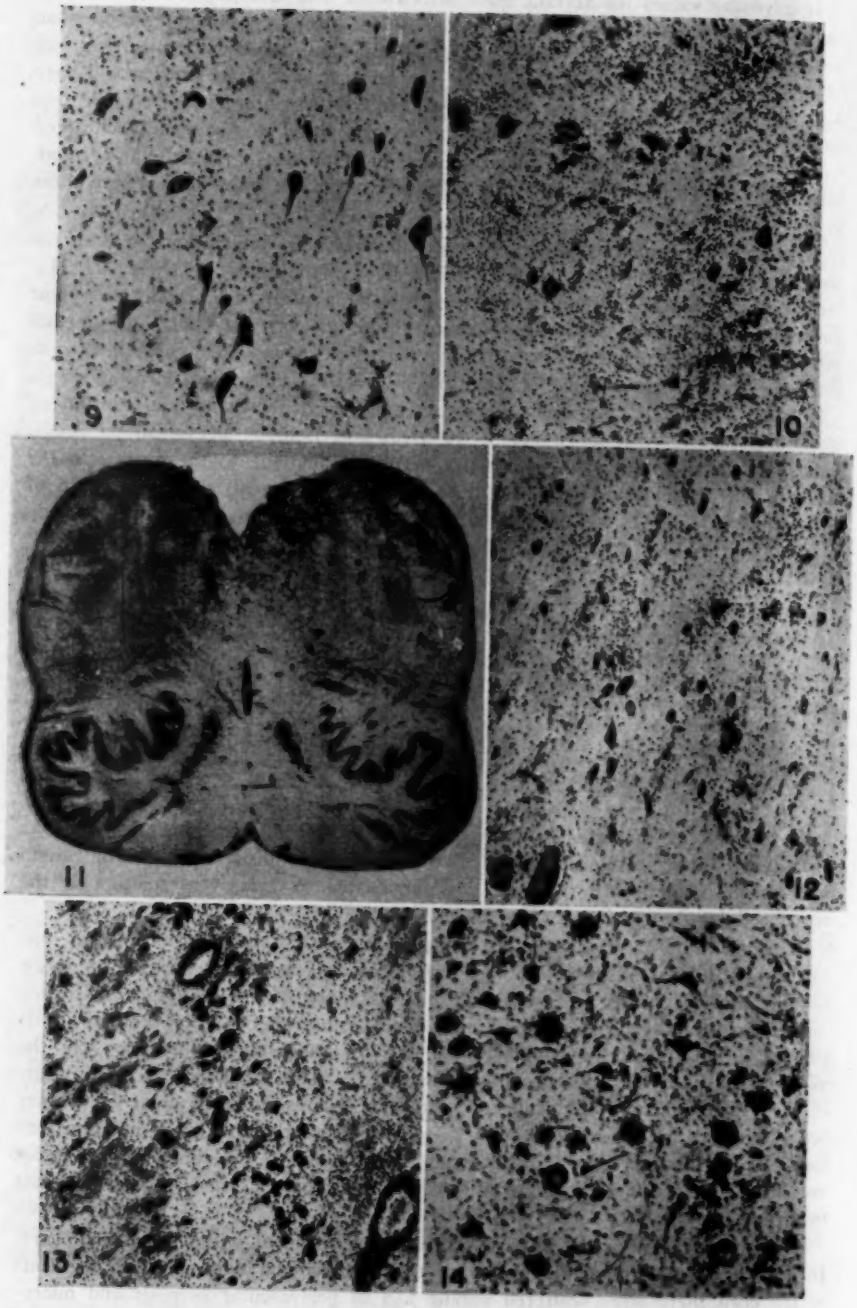
| | | | |
|---|---|---|---|
| 1. Cervical part of the cord | 28 | 8. Sacral part of the cord | 12 |
| 2. Medulla oblongata | 26 | 9. Subthalamus | 10 |
| 3. Pontile tegmentum | 22 | 10. Hypothalamus | 10 |
| 4. Thoracic part of the cord | 18 | 11. Dentate nucleus | 7 |
| 5. Red nucleus | 18 | 12. Motor area | 3 |
| 6. Lumbar part of the cord | 18 | 13. Thalamus | 3 |
| 7. Substantia nigra | 17 | 14. Lenticular nucleus | 2 |

their function as motor or sensory, are prominently affected, but the reticular formation is the most uniformly involved, usually by diffuse cellular infiltration. The nuclei ambigui are more or less implicated in every case, although the actual cellular destruction is only moderate.

An interesting observation is the tendency of the interstitial infiltrate to assume compact, nodular formations in the dentate nucleus (fig. 15). When the inferior olivary nucleus presents any significant intraparenchymal cellular infiltration (which is rarely encountered, in contradistinction to perivascular cuffing in its vicinity), the infiltrate is similarly nodular. These nodular formations in the olives were particularly noticed by Marinesco and co-workers[3] in their study of the 1929 Rumanian epidemic. Occasionally a conspicuous nodular focus may be encountered in the nuclei pontis, a region singularly free from inflammatory foci. Thus, when certain cellular groups giving rise to the cerebellar peduncles are involved, the physical aggregation of the intraparenchymal infiltration tends to assume a distinctive form.

Certain vagaries of the disease process may be noted within the spinal cord. In 1 case (2), in the white matter of the thoracic and upper lumbar segments of the cord there were scattered minute foci of perivascular necrosis and micro-

3. Marinesco, G.; Manicatide, M., and State-Draganesco: Ann. Inst. Pasteur **43**:223, 1929.




FIGURES 9 TO 14

*(See legends on opposite page)*

glial proliferation, a form of leukoencephalitis (fig. 16). In another case (1) there was more posterior than anterior poliomyelitis in the lumbar segments of the cord, and at the first sacral level posterior poliomyelitis was conspicuous in the absence of involvement of the anterior horns (figs. 17 and 18). Occasionally the inflammatory process was so severe, particularly in the upper cervical region of the cord, that small foci of rarefaction of the ground substance within the gray matter were produced, and pregitter cells were seen (cases 1 and 5, fig. 19). However, apart from such tiny foci no demyelination was encountered.

In 5 cases (2, 5, 9, 10, 13) of 9 in which the cord material was sufficiently complete at all levels to permit comparison, the newest process was found in the lumbar, sacral or lumbosacral segments of the cord. An inflammation was interpreted as being fairly recent when generous numbers of polymorphonuclear leukocytes were seen in the perivascular and interstitial exudates and participating in neuronophagia. This view is based on experimental studies.[4] In another case (3) there was progressive decrease in the severity of the inflammatory reaction from the cervical portion of the cord down, with the most recent lesions in the thoracic as opposed to the cervical part of the cord, and no lesions were found below the tenth thoracic segment. Therefore, if the hypothesis of spread via axonal channels is adopted, there was some evidence for an infection descending within the cord in 6 of 9 cases.

A general perusal of the evidence available from the topographic severity of the histologic lesions and from the clinical features leads me to classify the poliomyelitis in 3 cases (9, 11 and 12) as a primary spinal type with subsequent ascent of the inflammatory process, that in 9 as a primary "bulbar" type and that in (5) as indeterminate with respect to the general region of the neuraxis first affected by intraparenchymal inflammatory change.

## COMMENT

Since, unfortunately, blocks of the complete olfactory bulbs were available in less than half of the cases in this series, the negative findings as to involvement of these structures have correspondingly limited significance. Yet they are in accord with the similar essentially

4. Luhan, J. A.: Arch. Neurol. & Psychiat. **37**:479, 1937.

### Explanation of Figures 9 to 14

Fig. 9.—One plus or less involvement of the anterior gray matter of the cord. Cresyl violet; × 67.

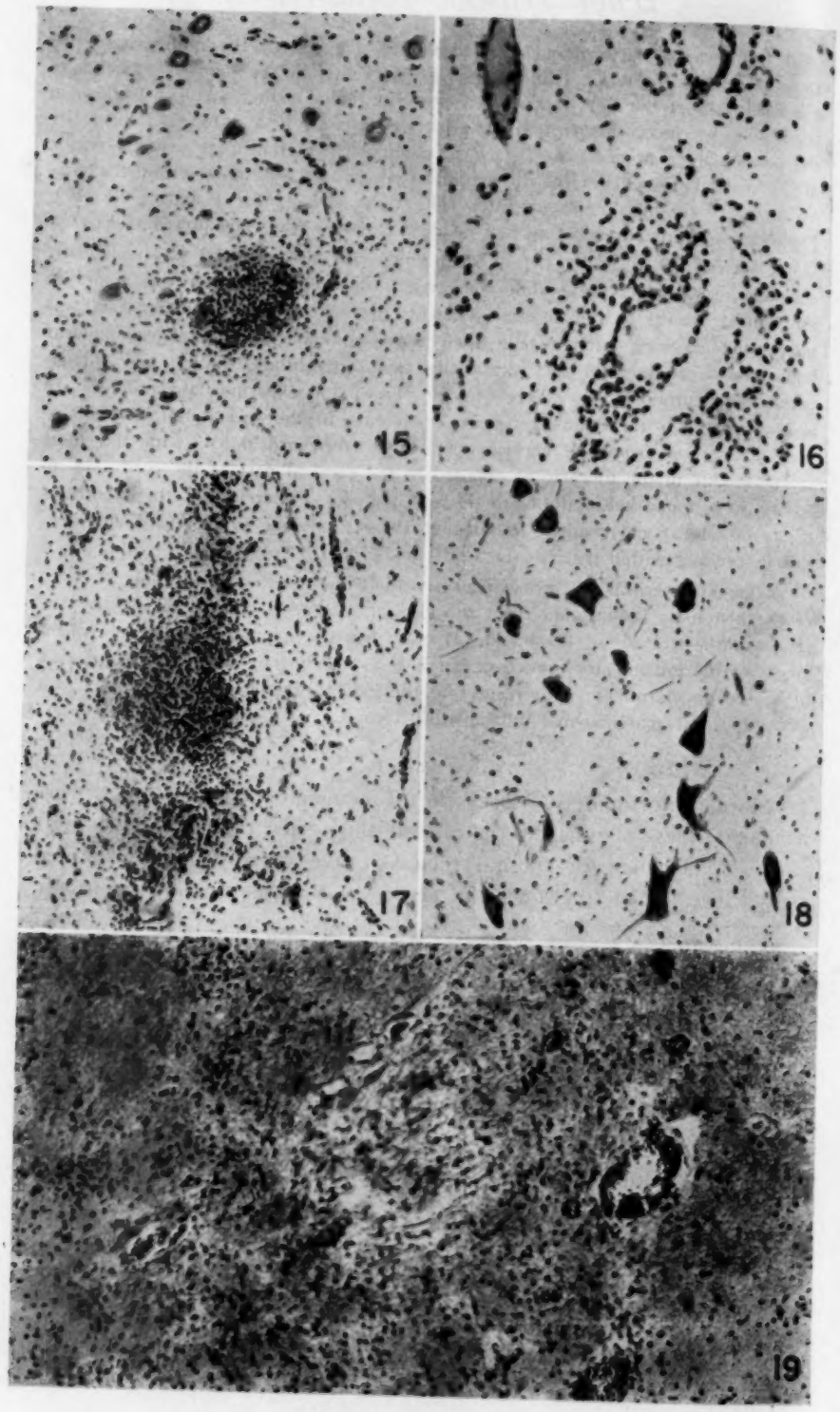
Fig. 10.—Three plus involvement of the anterior gray matter of the cord. Cresyl violet; × 67.

Fig. 11 (case 4).—Low magnification of the medulla oblongata which shows involvement graded 2½ plus (perivascular infiltrations, 3 plus; other inflammatory changes, 2 plus). Cresyl violet; × 3.6.

Fig. 12 (case 4).—Typical involvement of the substantia nigra. Cresyl violet; × 70.

Fig. 13 (case 6).—Dense inflammatory reaction in the region of the locus ceruleus of the pons. Cresyl violet; × 68.

Fig. 14 (case 6).—Neuronophagia within the facial nucleus. Cresyl violet; × 87.




FIGURES 15 TO 19

*(See legend on opposite page)*

negative histologic observations of previous investigators,[5] particularly Sabin,[5d] who worked with material from the 1937 epidemic. On the other hand, experimental poliomyelitis induced by intranasal instillation of the virus is regularly found to be represented by perivascular and interstitial infiltrative lesions of the olfactory bulbs.[6] Sabin and Ward[7] were unable to demonstrate the presence of the virus of poliomyelitis in the olfactory bulbs in 6 cases of human poliomyelitis. Howe and Bodian[2] demonstrated that in at least a hundred cases of experimental poliomyelitis in which the virus was introduced by the nasal route, the olfactory bulbs were involved by perivascular cuffing and interstitial infiltration, in contrast to the absence of involvement of the olfactory bulbs in fifty-odd cases in which inoculation was by some portal other than the intranasal. These workers and others showed that in the chimpanzee the olfactory tract is an available route but not the so-called natural one. The macaque, on the other hand, is susceptible to inoculation by the olfactory channel and refractory to inoculation by the oral or the gastrointestinal route. In an able review of the problem of the significance of the nasal pathway, King[8] stated, "It is possible that the relative importance of the nasal route in experimental poliomyelitis is purely an artificial condition, brought about by some degree of change in the virus and without necessary relation to the natural disease."

An interesting finding, confirming the work of a number of other investigators, is that the motor area, of all the cortical distribution,

5. (*a*) Horanyi-Hechst, B.: Deutsche Ztschr. f. Nervenh. **137**:1, 1935. (*b*) Swan, C.: Australian J. Exper. Biol. & M. Sc. **17**:345, 1939. (*c*) Robertson, E. G.: M. J. Australia **1**:156, 1940. (*d*) Sabin, A. B.: Am. J. Dis. Child. **60**:1313, 1940.

6. Sabin, A. B., and Olitsky, P. K.: J. A. M. A. **108**:21, 1937.

7. Sabin, A. B., and Ward, R.: J. Exper. Med. **73**:771, 1941.

8. King, L. S.: J. A. M. A. **113**:1940, 1939.

EXPLANATION OF FIGURES 15 TO 19

Fig. 15 (case 4).—Compact or nodular focus in the dentate nucleus. Cresyl violet; × 105.

Fig. 16 (case 2).—Perivascular microgliosis in the anterior paramedian white column of the first lumbar segment of the cord. Cresyl violet; × 245.

Fig. 17 (case 1).—Marked inflammatory reaction of the posterior gray column of the first sacral segment of the cord. (Higher magnification reveals the presence of numerous polymorphonuclear cells.) Cresyl violet; × 90.

Fig. 18 (case 1).—Tissue from the anterior horn region of the same segment as the tissue shown in figure 17, revealing normal appearances. Cresyl violet; × 90.

Fig. 19 (case 1).—Area of rarefaction in the anterior gray matter of the cervical part of the cord; diffuse type of cellular infiltration. Hematoxylin and eosin; × 125.

appears selectively vulnerable to the depredations of the virus. Involvement of the motor cortex in a case of human poliomyelitis was described in 1929 by André Thomas and L'Hermitte[9]; in that year Hurst[10] published his report of comprehensive studies on the histologic aspects of experimental poliomyelitis, in which he pointed out that only in the motor area of all the cerebral cortex do the neurons succumb to the infection. Pette and his co-workers[11] substantiated Hurst's observation in their exhaustive studies of experimental poliomyelitis, published in 1932; they found that the inflammatory process was practically limited to the third and fifth layers within the motor area. Spielmeyer[12] in 1932 found the motor cortex implicated in 7 of 8 cases of human poliomyelitis and called attention to the selective involvement of the motor area in this disease. Kornyey[13] (1933) reported that 8 human cases of poliomyelitis studied for distribution of the pathologic changes of the brain all showed lesions limited to the precentral area. Stiefler and Schenk[14] described involvement of the motor area in 7 of 9 instances of human poliomyelitis in which the anterior central gyrus was examined. Horanyi-Hechst[5a] in 1935 found lesions in the precentral area in 19 of 24 human cases, with 3 showing lesions in the caudal part of the frontal agranular cortex also. Swan[5b] in a study of 8 cases of human poliomyelitis reported in 1939 found the area gigantopyramidalis of Brodmann involved in all, and with a single exception (lesions in the cornu ammonis) this was the only area of the cortex affected. Howe and Bodian[2] noted involvement of the motor cortex in 12 of 13 cases of human poliomyelitis; these lesions consisted of perivascular cuffing, neuronophagia and focal mesodermal glial infiltrations in all layers but especially in the layer of Betz cells. In 2 cases lesions were shown in area 6; in 4, in area 1, and in 2 there were infrequent lesions in the frontal granular cortex. That the virus may be recovered from the motor area in human poliomyelitis was demonstrated by Sabin and Ward.[7]

These reports together with the observations in the present study indicate that involvement of the motor area in the form particularly of interstitial mesodermal-glial infiltrations may be found in almost 90 per cent of cases of human poliomyelitis. This involvement, furthermore, is selective in that either the motor area alone of all the cortex is involved or it is the most severely implicated of all the cytoarchitectural regions. In this study most of the sections of the motor area and of its

9. André Thomas and L'Hermitte, J.: Rev. neurol. **1**:1242, 1929.

10. Hurst, E.: J. Path. & Bact. **32**:457, 1929.

11. Pette, H.; Demme, L., and Kornyey, St.: Deutsche Ztschr. f. Nervenh. **128**:125, 1932.

12. Spielmeyer, W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **142**:159, 1932.

13. Kornyey, St.: Ztschr. f. d. ges. Neurol. u. Psychiat. **146**:724, 1933.

14. Stiefler, G., and Schenk, E.: Deutsche Ztschr. f. Nervenh. **130**:68, 1933.

vicinity were from the upper third (including that of the paracentral lobule), where the Betz cells are particularly numerous and easy to identify. Kornyey [13] reported that the pathologic changes were the most pronounced in the upper third of the anterior central gyrus and were usually lacking in the lower third. Horanyi-Hechst [5b] found that the center for control of the leg was most severely involved as a rule. Swan [5b] did not find any consistent difference in the severity of the lesions in different areas of the motor cortex in his series; in 1 case the lower half of the gyrus was most heavily implicated. For the pathologist the practical significance of the relatively selective involvement of the gigantopyramidal motor area (FA$\gamma$ of von Economo and Koskinas) is that this peculiarity of distribution constitutes an aid to the presumptive identification of the disease. The Betz cell area, moreover, in practice is easily recognized.

The uniform occurrence of severe involvement of the reticular formation in the medulla in itself suggests that this may be an important factor in the fatal outcome of acute poliomyelitis.

There was no demyelination except in 2 cases in which there were foci of rarefaction of the ground substance of the gray matter of the cervical part of the cord, as previously mentioned; but in this series of cases the disease was acute. In chronic cases some demyelination may be found in the cord (Peers [15]) and even cyst formation in the gray matter, as in the case of a 26 year old woman who had been living in a respirator for six hundred and thirty-seven days (Baker [16]).

Whether or not the primary attack is on susceptible nerve cells and the inflammatory reaction is a secondary consequence of the chemical interaction of virus and ganglion cell has not been conclusively answered. Howe and Bodian [2] have adduced experimental evidence that living nerve cells are necessary for the production of typical lesions. They demonstrated absence of inflammatory reaction in the thalamus of an inoculated animal in which all nerve cells had previously disappeared from this structure after destruction of their axonal terminations in the cerebrbal cortex. Yet in poliomyelitis, whether experimental or human, the histologic changes show a disturbing lack of strict parallelism in topography and severity between the manifest disease or destruction of ganglion cells, the intraparenchymal mesodermal-glial reaction and the (mild) leptomeningeal infiltrations.

Although there is some evidence that the virus is disseminated within the neuraxis by axonal channels (for example, infection descending within the cord in 6 of 9 cases), it is not inconsistent with the hypothesis that the virus is not strictly neurotropic. For a virus to propagate only

15. Peers, J. H.: Am. J. Path. **19**:673, 1943.
16. Baker, A. B.: Journal-Lancet **64**:224, 1944.

in nervous tissue and to seek immurement within the secluded neuraxis would mean its biologic suicide. There has been much experimental work within recent years to indicate that the virus may be found in the human alimentary tract, and the presumption follows that it may propagate there or at least outside the nervous system. Whether the hyperplasia of mesenteric and intestinal lymph nodes frequently observed in poliomyelitis bears some relation to the portal of entry of the virus was considered in this series. The most severe intestinal lymphatic hyperplasia (graded 3 to 4 plus) occurred in cases 1, 2 and 3, in which the infection was considered to be of primary "bulbar" type, although abdominal pain preceded the bulbar symptoms in case 1. The patient in case 3 (a man 20 years old) had no involvement of the olfactory bulbs, changes graded 3 plus in the medulla oblongata, 2½ plus in the cervical part of the cord, 1 plus in the upper thoracic region of the cord and no lesions in the spinal cord below the tenth thoracic segment. In the remaining 3 cases there was moderate intestinal lymphoid hyperplasia; in 2 of these (11 and 12) the involvement was clinically of the spinal type in the beginning, with intercostal paralysis, and in the remaining case (5) the disease process was not easily classifiable with respect to initial neuraxial localization. In 1 case (9) of primary spinal poliomyelitis there was no significant hyperplasia of lymph nodes at necropsy.

## SUMMARY

The pattern of distribution of the histologic lesions of poliomyelitis was studied in 13 fatal cases which predominantly were instances of the "bulbar" type of the disease. These cases occurred in the 1943 Chicago epidemic.

Involvement of the gigantocellular motor cortex was found in 12 of the 13 cases. This observation is in conformity with the findings of several previous investigators and argues for the presence of a "system factor" in poliomyelitis. It is also a clue to the identification of the virus so far as this may be ventured from the exhibition of pathologic changes in the central nervous system.

The olfactory route was probably not the important channel of inoculation in these cases.

Certain caprices of histologic reaction may be encountered in human poliomyelitis.

# PRIMARY HYPERPARATHYROIDISM

## A Report of Five Cases that Exemplify Special Features of this Disease (Infarction of a Parathyroid Adenoma; Oxyphil Adenoma)

EDGAR H. NORRIS, M.D.
Resident Consultant, Army Institute of Pathology
WASHINGTON, D. C.

PRIMARY hyperparathyroidism is the condition that results from adenoma of the parathyroid glands. Characteristically in the majority of cases the associated clinical findings are hypercalcemia, hypophosphatemia, osteitis fibrosa cystica generalisata of varying degrees and/or renal parenchymal calcification and renal lithiasis. All of the associated abnormalities are effects produced by the excess of parathyroid hormone generated by the adenoma. Since the disease is due to a hypersecreting neoplasm of the parathyroid glands, it is quite properly designated by the term "primary hyperparathyroidism." This designation further serves to distinguish it from those states in which hypersecretion and hyperplasia of the parathyroid glandules result from, and are therefore secondary to, such prefatory conditions as chronic renal insufficiency.

Through the courtesy of Colonel J. E. Ash and Colonel B. Lucke,[1] I have been privileged to study the cases of parathyroid adenoma among the material collected at the Army Institute of Pathology in Washington, D. C. Because 5 of these cases exemplify many typical characteristics and a number of important special features of primary hyperparathyroidism they are reported in this communication.

### REPORT OF CASES

CASE 1.—*Clinical Summary.*—A white man 21 years of age, a technical sergeant, was found on roentgenologic examination to have multiple osteolytic lesions involving the skull, the eleventh rib, the pelvis and the left femur. These skeletal lesions were asymptomatic. A segment of the eleventh rib was removed for diagnosis. Grossly the medulla of the rib was filled with soft brown tissue. This was surrounded by a thin cortex of brittle bone. Microscopically, the brown medullary tissue was made up of fibroblasts and multinucleated giant cells; some areas showed a tendency toward cyst formation. The pathologic diagnosis of osteitis fibrosa cystica was supported by chemical studies of the blood for calcium, phosphorus and phosphatase. At operation, Nov. 11, 1943, the neck was explored, and an adenoma of the left inferior parathyroid gland and also the normal right inferior parathyroid gland were removed.

From the Department of Anatomy of the Washington University School of Medicine, St. Louis, the Army Institute of Pathology, Washington, D. C., and the Division of Research in the Medical Sciences of the Lynn Clinic, Detroit.

*Description of Specimens.*—The tumor from the region of the left inferior parathyroid gland was an irregular encapsulated body made up of soft brown tissue (fig. 1). It measured 3.0 by 1.7 by 1.5 cm. and weighed 3.9 Gm. The capsular surface was smooth and gray; the cut surface was brown and seemingly uniform in structure.

The tissue removed from the region of the right inferior parathyroid gland was a small brown body measuring 0.65 by 0.3 by 0.3 cm. and weighing 0.05 Gm. (fig. 1).

Microscopically, the tumor had a monotonously uniform structure (fig. 2). The parenchyma consisted of closely packed epithelial cells arranged in irregular blocks that were separated by a delicate fibrous stroma containing many capillaries and sinusoids and that produced no particular type of pattern. No follicles or colloid deposits were seen. The parenchymal elements were nearly all dense cells,[1] clearly outlined, polyhedral, of moderate size, with a finely granular, faintly azurophil cytoplasm and relatively large, centrally placed nuclei (fig. 2). A perinuclear halo was present in a few cells which had the characteristics of small vesicular cells.

*Pathologic Diagnosis.*—Parathyroid adenoma. The glandule removed from the right side was a normal parathyroid gland.

CASE 2.—*Clinical Summary.*—A white man 23 years of age had had scarlet fever and mumps at 14 and pneumonia at 17. Four years previously, in 1939, he had passed a small urinary calculus. In August 1943 he complained of pain in his back and of sluggishness; a tremor developed, and he became restless. Roentgen examination disclosed multiple parenchymal calcifications in both kidneys. The long bones, especially the upper end of the right femur, the pelvis and the skull were markedly decalcified and cystic.

During the last days of October 1943 the following laboratory data were assembled: The hemoglobin content of the blood was 12.2 Gm. per hundred cubic centimeters. Red cells numbered 4,170,000 per cubic millimeter; white cells, 8,550, with polymorphonuclears 63 per cent, lymphocytes 34 per cent, eosinophils 2 per cent and monocytes 1 per cent. The serum calcium was 18.7 mg. per hundred cubic centimeters; the phosphatase was 22.1 Bodansky units. The urea nitrogen of the blood amounted to 17.6 mg. per hundred cubic centimeters. The urine contained albumin (3 plus) and many white blood cells.

Nov. 8, 1943 an adenoma was removed from the region of the left lower parathyroid gland. Following the operation, despite treatment, the blood calcium dropped rapidly to 7.2 mg. in three days, but gradually rose thereafter to normal.

*Description of Specimen.*—The tumor was an elongated ovoid mass measuring 2.2 cm. in length by 1.0 cm. in its greatest width and weighing 1.72 Gm. The surface was covered by a thin, rather opaque whitish capsule. On the cut surface the tumor was seen to be divided by a fibrous septum into two nodules; the neoplastic tissue was soft and presented a homogeneously pale, yellowish brown appearance.

Microscopically, the tumor was made up of irregular masses of closely packed epithelial cells, these parenchymal masses being outlined and separated by an extremely delicate vascular stroma (figs. 3, 4 and 6). Although, as will be seen, there was some variation of the morphologic picture in different parts of the neoplasm, the most characteristic pattern was that illustrated in figure 3. The large majority of the parenchymal elements were typical vesicular cells[1]; they

1. Norris, E. H.: The Parenchymal Cytological Elements of the Human Parathyroid Glands, to be published.

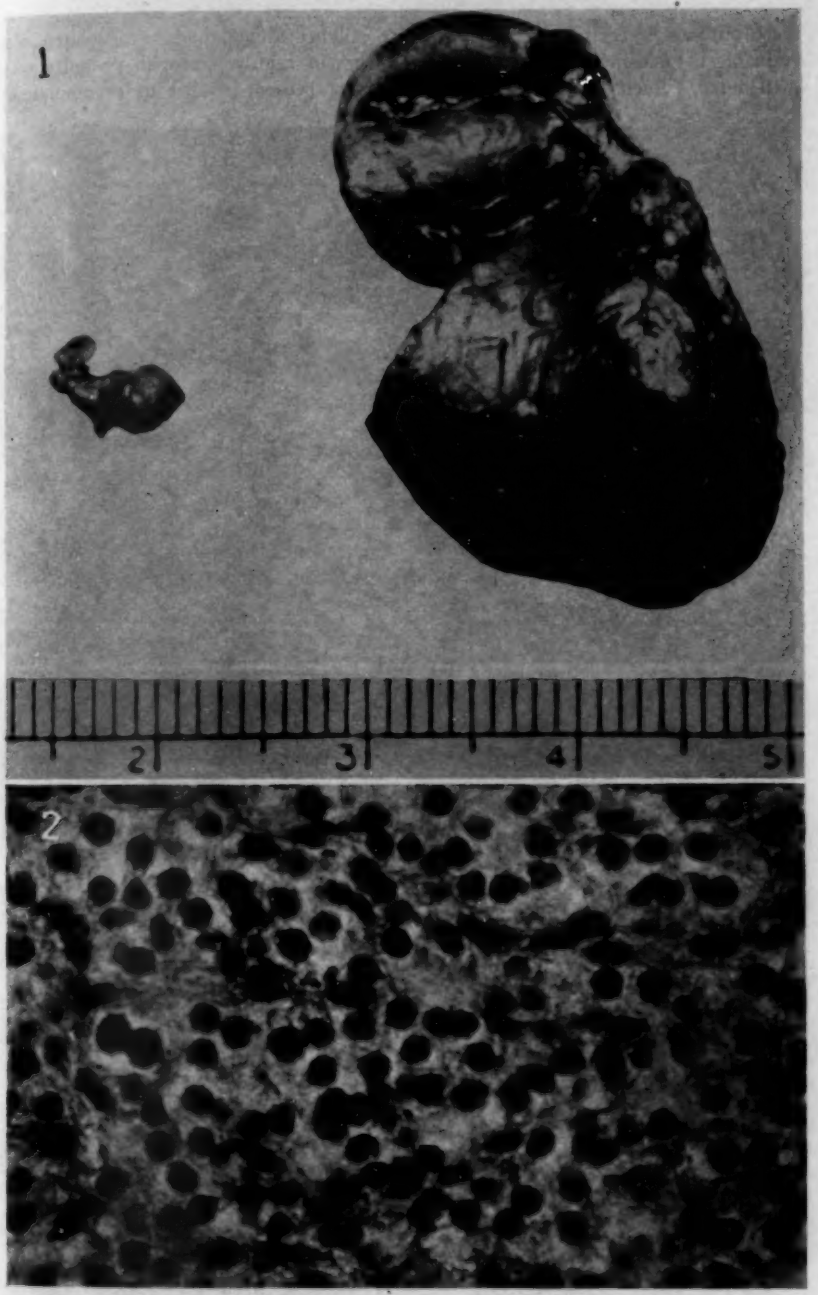



Fig. 1.—Photograph of the gross specimens removed in case 1. The small mass is the normal parathyroid gland and the larger is the adenoma.

Fig. 2.—Photomicrograph of a typical area from the adenoma of case 1. Note the monotonous character of the tumor. Nearly all of the cells are dense cells; only an occasional primordial or small vesicular cell is seen. × 700.

were outlined by distinct cell walls, were polyhedral and had a vacuolated nonstainable cytoplasm in which variable numbers of faintly azurophil granules were distributed. Such stainable cytoplasm as was present tended to accumulate at

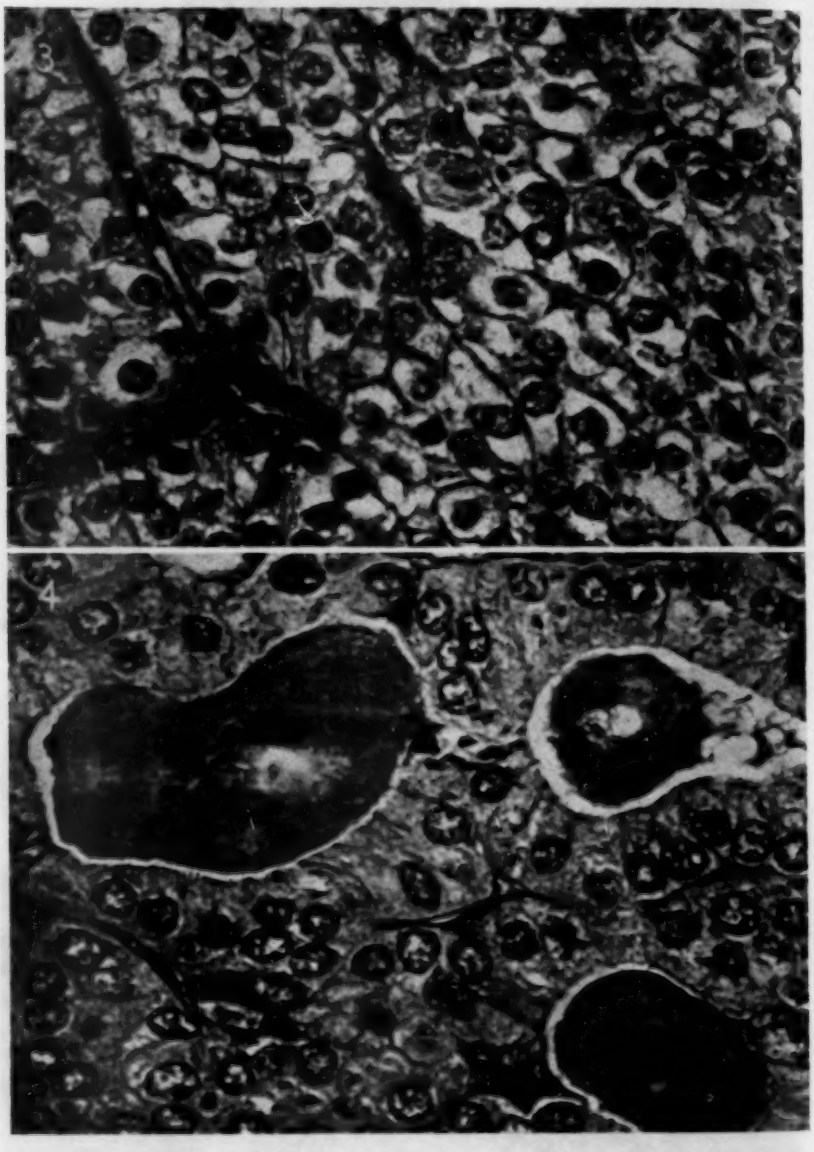



Fig. 3.—Photomicrograph of an area typical of the greater part of the adenoma of case 2. Nearly all of the cells are vesicular cells; only an occasional primordial or dense cell is seen. × 700.

Fig. 4.—Photomicrograph of a selected area in the adenoma of case 2 to illustrate an unusual structural pattern. Note the colloid-filled parathyroid follicles. Most of the cells are columnar dense cells; a few cells in the right upper corner may be called columnar vesicular cells. × 700.

the periphery of the cell, leaving a clear halo about the nucleus. The nuclei were relatively large, more or less round bodies surrounded by a thin but distinct nuclear membrane, and they contained a moderate amount of finely granular chromatin and prominent nucleoli (fig. 3).

In some parts of the tumor the cells were arranged so as to form follicles (fig. 4). The follicular lumens were more or less filled with eosinophilic hyaline colloid. Here and there some smaller droplets of colloid were found outside of follicles in the stroma near vascular elements. The cells of the follicular walls were columnar in form, and their rounded nuclei were located in the bases of the cells away from the lumen. Except that these particular cells had departed from the polyhedral form, their characteristics were typically those of dense or vesicular cells.

In still another part of the tumor, subjacent to the capsule, considerable numbers of large clear cells were found (figs. 5 and 6). These cells had distinct walls and were polyhedral in form; their cytoplasm, which contained relatively few granules, was nearly nonstainable, and their nuclei were relatively small and pyknotic. As is apparent in figures 5 and 6, all manner of transitional forms were found between the vesicular cells and the large clear cells.[1]

The capsule and the large stromal septums were composed of dense fibrous tissue. As illustrated in figure 7, nests of epithelial cells were found within this capsular tissue. Although such a picture suggests invasion, in the absence of other evidences of cancer, it is better interpreted as a marginal portion of the neoplastic parenchyma extending into the tissue spaces of the capsule.

*Pathologic Diagnosis.*—Parathyroid adenoma.

CASE 3.—*Clinical Summary.*—A white woman 48 years of age, a housewife, was first observed from July 7 to July 21, 1944. She complained of nervousness, fainting spells and slight convulsive attacks and called attention to a cervical swelling that had been present for some months. She stated that she had been well until June 25, 1944, when she suffered a severe convulsion at the onset of the present illness. Physical examination revealed a small mass in the neck at the level of the thyroid cartilage.

The following laboratory data were assembled: The hemoglobin content of the blood was 10 Gm. per hundred cubic centimeters. Red cells numbered 3,740,000; white cells, 9,500, with lymphocytes 30 per cent, polymorphonuclears 68 per cent and monocytes 2 per cent. The blood creatinine amounted to 1.95 mg.; the urea nitrogen, to 24.1 mg. The serum calcium was 5.0 mg. per hundred cubic centimeters. The urine was normal. The rate of the basal metabolism was —7 per cent.

The patient was given large amounts of calcium gluconate with benefit. July 14, 1944 parathyroidectomy was performed.

*Description of Specimen.*—The adenoma was removed together with a part of the lateral lobe of the thyroid gland, within which it was partially embedded. The adenoma was well encapsulated and measured 2.5 by 2.0 by 0.8 cm. On section the tumor was yellowish in color and almost caseous in consistency.

Microscopically, the major part of the tumor was made up of the shadow-like coagulated remains of epithelial cells that together constituted a necrotic mass. Under low power the cellular debris was seen arranged in closely related irregular blocks or strands—not unlike the usual pattern of a parathyroid adenoma (fig. 8). The capsule was composed of rather dense fibrous tissue, between the layers of which were elongated islands of uninjured parathyroid tissue (fig. 8). As illustrated in figure 9, these parenchymal islands contained a majority of clear cells

with smaller numbers of vesicular and primordial cells. On the inner surface of the capsule and adjacent to the necrotic material there was some recent fibroblastic proliferation; some of these fibroblasts and a few more leukocytes had invaded the periphery of the necrotic mass. Considerable numbers of pigment-laden macrophages were seen in the looser outer layer of the capsule.

*Pathologic Diagnosis.*—Parathyroid adenoma with recent infarction.

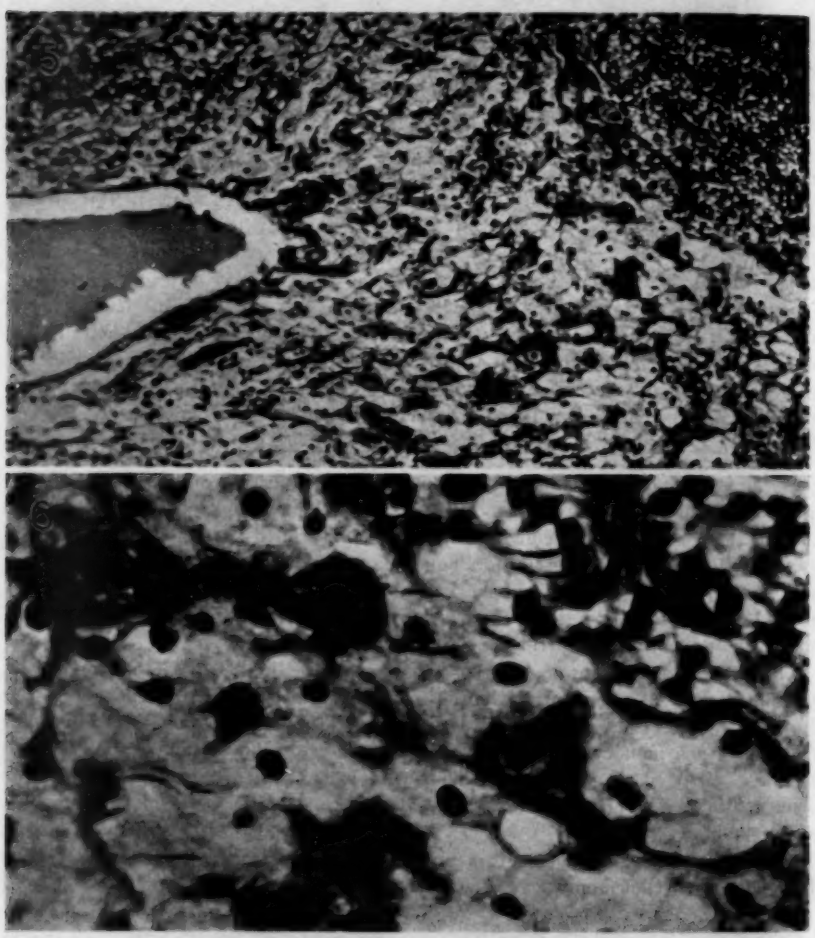


Fig. 5.—Photomicrograph of a subcapsular region in the adenoma of case 2. The majority of the cells in this area are large and small clear cells, with some vesicular and a few dense cells in the left upper corner. × 150.

Fig. 6.—Photomicrograph of a small field taken from near the center of the region pictured in figure 5. Large and small clear cells are in the majority; some vesicular cells are noted in the right upper corner. × 700.

Case 4.—*Clinical Summary.*—A white man 27 years of age, a sergeant in a supply and service company, came under observation, May 3, 1944, because of a tumor in the right side of the mandible which had been noticeable for about six months. The mandibular lesion, excised May 6, was made up of brownish

tissue containing spicules of bone. Microscopically, the tissue consisted of loose cellular connective tissue in which numerous multinucleated giant cells were dispersed. A diagnosis of "benign giant cell tumor of the mandible" was made.

About a year later the patient was admitted to the urologic service because of pain in the region of the right kidney. He stated that on the average at least one renal stone had been passed every year since 1927 (eighteen years). The last stone was passed in May 1944, and since then there had been intermittent pain in the region of the left kidney. Roentgenologic examination revealed dysfunction of both kidneys with diffuse calcification.

The following laboratory data were assembled: The serum calcium amounted to 14.0 mg. and the phosphorus to 1.8 mg. per hundred cubic centimeters; the phosphatase value was 8.0 Bodansky units. A twenty-four hour specimen of the urine, 2670 cc., contained 19.5 mg. of calcium per hundred cubic centimeters.

May 17, 1945 an adenoma was removed from the region of the left upper parathyroid gland. The postoperative course was excellent. The serum calcium dropped quickly to 10 mg. per hundred cubic centimeters, and the calcium content of the urine became normal.

*Description of Specimen.*—The tumor was a rather soft, well encapsulated mass that measured 2.6 by 1.1 by 0.6 cm.; the tissue had a tannish gray color.

Microscopically, the tumor had a uniform structure. With one exception its constitution was similar to that of the neoplasm observed in case 1; in the parenchyma dense and vesicular cells were about equally numerous.

*Pathologic Diagnosis.*—Parathyroid adenoma.

CASE 5.—*Clinical Summary.*—A white woman 23 years of age, a Women's Army Corps clerk, noticed a lump on the right side of her neck about June 1, 1945. It caused no trouble except when she wore a tight collar or when she tried to swallow a large mouthful of food or fluid. No other symptoms were noted except that the last two menstrual cycles had changed from "28—4 day type" to a "28—1 or 2 day type."

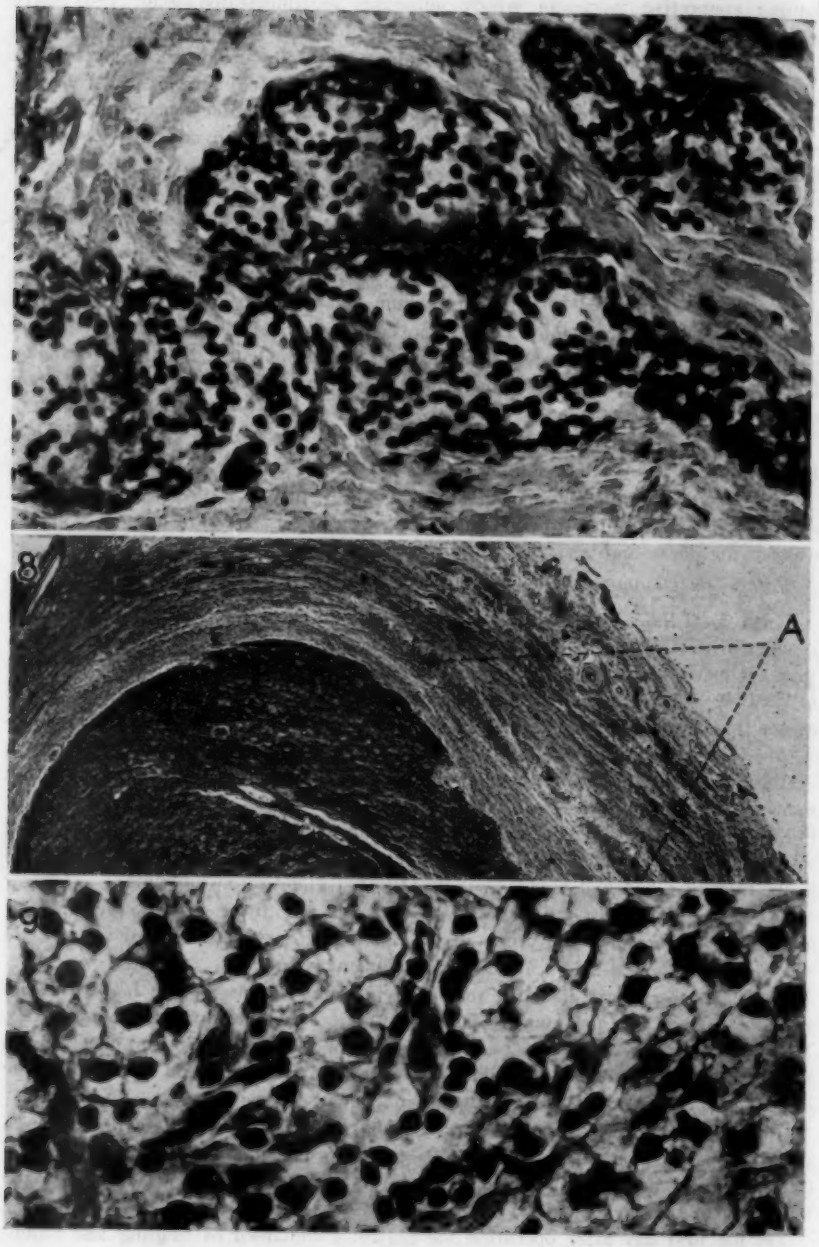
But for the swelling in the right side of the neck the physical examination showed no abnormality. In the lower lateral portion of the neck just anterior to the anterior border of the right sternocleidomastoid muscle was a small round smooth mass, approximately 4 cm. in diameter. The mass seemed to be attached to the thyroid gland and moved with the thyroid gland on swallowing.

Roentgenographically, the chest was normal, and there was no evidence of tracheal deviation or of substernal thyroid gland. Roentgenograms of the skeletal system revealed no decalcification or cystic changes.

The laboratory data included the following observations: The blood calcium amounted to 12.3 mg. per hundred cubic centimeters June 12 and 12.8 mg. June 28. The basal metabolic rate was —13 per cent June 14 and —10 per cent July 9. Serologic tests revealed no syphilis. The urine, the red blood cell count, the white blood cell count and the hemoglobin content were normal.

July 12 (four weeks after the cervical mass was first noticed by the patient) she was operated on. An adenomatous tumor occupied all of the right lobe of the thyroid gland except the upper pole; therefore, subtotal resection of the right lobe of the thyroid gland was done.

On the first day after operation the patient complained of tingling and numbness of the fingers and the toes and showed hyperirritability of the facial nerves; otherwise the postoperative course was normal. August 13 the blood calcium was 10.2 mg. and the blood phosphorus 3.5 mg. per hundred cubic centimeters. The blood phosphatase value was 9.1 units.




FIGURES 7, 8 AND 9

*(See legend on opposite page)*

*Description of Specimen.*—The tumor consisted of a well encapsulated nodule 3.5 cm. in diameter, partially surrounded by thyroid tissue.

Microscopically, the tumor had a uniform structure (fig. 10). The parenchyma was composed of sheets of closely packed epithelial cells. The stromal elements and the vascular channels were delicate and scattered in such a way as to divide the epithelial tissue into blocks and masses of irregular form. An occasional droplet of extrafollicular colloid was noted, but there were no follicles.

Nearly all of the cells were of one type—relatively large, with distinct walls. As compared with typical primordial or dense cells, many in this tumor had preserved their polygonal form, but there was a definite tendency toward reduction in the number of angles and replacement of straight sides by smoothly curving outlines. In other words, these cells approached forms that were more

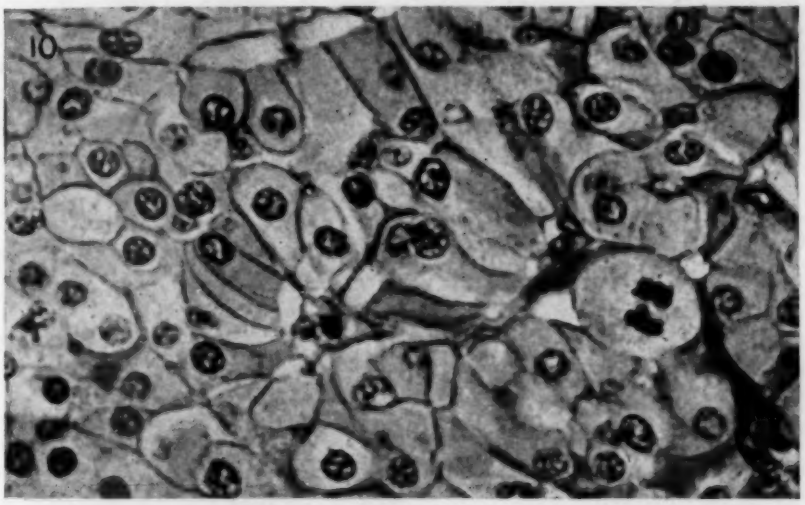



Fig. 10.—Photomicrograph of a typical area from the adenoma of case 5. Practically all of the cells in this tumor are large pale oxyphil cells. × 661.

nearly quadrangular than polyhedral, with gentle curves replacing sharp angles. The cytoplasm was made up of evenly distributed fine eosinophilic granules—in some cells, a bit more coarse. An occasional mitosis was seen. Although in

EXPLANATION OF FIGURES 7, 8 AND 9

Fig. 7.—Photomicrograph of nests of parenchymal cells that have extended from the adenoma into the tissue spaces of the dense connective tissue that makes up the capsule of the adenoma. × 354.

Fig. 8.—Photomicrograph of a section from the adenoma of case 3. Note the complete necrosis of the main body of the adenoma, and the surrounding uninjured capsule. The leaders marked *A* indicate two wedge-shaped islands of uninjured parathyroid parenchyma lying between layers of the capsule. × 19.

Fig. 9.—Photomicrograph of an area from one of the capsular islands shown in figure 8. These parenchymal cells are apparently uninjured and are good examples of vesicular and clear cells. × 661.

certain areas a few vesicular and dense cells were present, the majority of the cells of this tumor were morphologically like the large pale oxyphil cells of normal glandules (fig. 10).

*Pathologic Diagnosis.*—Parathyroid adenoma.

## COMMENT

De Santi [2] in 1900 and Benjamins [3] in 1902 are often credited with having been the first to describe primary tumors of the parathyroid glands; however, in the light of present knowledge their descriptions are not convincing. De Santi did not report on the histologic aspects of the tumor which he observed. The tumor Benjamin described was as large as a child's head! And despite the fact that both these tumors were large, neither author recorded skeletal changes. Other observers of the same period (Kocher,[4] Langhans [5] and de Quervain [6]) recorded cases of cervical tumor that may have arisen from the parathyroid glands, but again the histologic evidence of origin is not clear. During the first three decades of the present century there appeared scattered reports of parathyroid tumors found at autopsy. Probably the earliest record of a case that may be accepted today as one of parathyroid adenoma was that preesnted by Erdheim [7] in 1903. A year later Askanazy [8] was the first to call attention to the association of a parathyroid tumor and skeletal lesions. Thereafter the association of an enlarged parathyroid gland and osteitis fibrosa cystica generalisata was recognized, but it was not until the first time that an enlarged parathyroid gland was surgically removed, by Mandl [9] in 1925, that widespread practical interest was stimulated. Since 1925 there has been a rapidly growing list of case reports, mostly from surgical clinics. Norris [10] in a recent survey of the literature showed that more than 300 cases of parathyroid adenoma have been reported. However, because no one investigator has had the opportunity to study more than a few cases, it is not surprising that fundamental and practical knowledge of primary hyperparathyroidism has developed gradually.

The 5 cases reported in this paper exemplify features to which special attention may be called. Certain of these features are of practical clinical and pathologic interest. In addition, it must not be for-

2. De Santi: Internat. Centralbl. f. Laryng. u. Rhin. **16**:546, 1900.
3. Benjamins, C. E.: Beitr. z. path. Anat. u. z. allg. Path. **31**:143, 1902.
4. Kocher, T.: Virchows Arch. f. path. Anat. **155**..532, 1899.
5. Langhans, T.: Virchows Arch. f. path. Anat. **189**:69, 1907.
6. DeQuervain, F.: Deutsche Ztschr. f. Chir. **100**:324, 1909.
7. Erdheim, J.: Beitr. z. path. Anat. u. z. allg. Path. **33**:158, 1903.
8. Askanazy, M.: Arb. a. d. path. Inst. zu Tubingen **4**:398, 1904.
9. Mandl, F.: Wien. klin. Wchnschr. **38**:1343, 1925.
10. Norris, E. H.: The Parathyroid Adenoma, Surg., Gynec. & Obst., to be published.

gotten that the careful study of such cases as are reported in this communication offers another important potentiality and much needed facility; the possibility of analyzing the functions of the different kinds of parathyroid cells in correlation with their histologic structure and chemical activity in pathologic states promises an advance toward the understanding of their normal physiologic and metabolic functions.

Case 1, case 2 and case 4 present features that may be compared and contrasted. In each the adenoma was single and of moderate size and was associated with hypercalcemia and the characteristic lesions of osteitis fibrosa cystica generalisata. In addition, case 2 and case 4 were complicated by renal lithiasis and calcification. There can be no doubt that dense cells are secretorially active, for the adenoma of case 1 was composed almost entirely of cells of this type. In the tissue in case 4 dense cells and vesicular cells were about equally numerous. In contrast, the adenoma of case 2 has a great preponderance of vesicular cells, and the serum calcium was found at a high level: here too, however, there is no room for doubting that vesicular cells are secretorially active. Nevertheless, two questions are posed. Elsewhere [1] I have expressed my opinion that vesicular cells are functionally more active than dense cells. Can this opinion be supported by the regular finding of higher serum calcium levels in those cases in which the adenoma contains a preponderance of vesicular cells? Is it in those cases of primary hyperparathyroidism in which the higher levels of serum calcium are observed that renal lithiasis and calcification tend to occur? Although it seems likely that ultimately both queries may be answered in the affirmative, the data recorded for these 5 cases are insufficient and the information to be found in the literature is inadequate to establish the concepts. To increase their value, future case reports should contain a careful and complete record of the information needed to enable exhaustive analysis of groups of reported cases.

In 1935 Castleman and Mallory [11] presented an excellent pathologic study of the parathyroid gland as observed in cases of hyperparathyroidism and they differentially delineated the features of adenoma and hyperplastic glands. These authors stated:

> . . . the histological picture of the hyperplastic gland, at least of the more common wasserhelle type, is so characteristic, so different from anything we have seen in the cases of single tumor formation that we believe a diagnosis of hyperplasia should be possible as a rule from the histological examination of a single gland, even from a frozen section during an operation. The uniform, giant sized clear cells, the acinar arrangement, the basal orientation of the nuclei form a readily recognizable picture.

In general my experience agrees with the statement quoted. However, as in cases 2, 3 and 4, I have seen sufficient exceptions to the rule to

11. Castleman, B., and Mallory, T. B.: Am. J. Path. **11**:1, 1935.

caution against too broad generalization. The variety of cell types and structural patterns illustrated in figures 3, 4, 5, 6 and 7 and all found in the adenoma of case 2 are considerable.

The striking absence of oxyphil cells in the adenoma in cases 1, 2 and 4 is a point worthy of attention. This observation accords with the findings in the majority of the cases of adenoma that have been described. I interpret the absence of oxyphil cells to signify two things. First, if oxyphil cells were present in the gland prior to the development of the adenoma, they must have reverted to the cell type from which they were derived. Second, oxyphil cells are probably concerned with some other function than that carried on by the primordial, vesicular, clear, dense, and dark cells. The adenoma of case 5 was true oxyphil adenoma, and it is important to note that the only clinical symptom was a cervical swelling; the patient had no skeletal or renal symptoms or signs, and there was little elevation of the serum calcium. A nearly identical case was reported by Cope [12] in 1944; the patient had a nodule in one side of the thyroid gland which turned out to be an oxyphil adenoma. Cope stated:

> A parathyroid tumor was not suspected preoperatively and the chemical studies necessary to exclude hyperfunction were, therefore, not made. It is probable, however, since there was no clinical tetany after removal of the tumor, that hyperfunction did not exist. This belief is strengthened by Castleman's opinion that the presence of pale oxyphil cells in large numbers in an adenoma indicate nonhyperfunction.

In 1938 McQuillan [13] reported a similar case of oxyphil adenoma; the patient suffered only from pressure symptoms (dysphagia) due to a cervical tumor, and the bones and the blood chemistry were found to be normal.

The prominence of oxyphil cells in the normal parathyroid glands of adults being kept in mind, the inconspicuousness of these cells in most cases of parathyroid adenoma and the picture presented in cases such as these 3 should guide in determining the function of oxyphil cells.

In the adenoma of case 2 the presence of areas of clear cells bespeaks a high degree of functional activity for the tumor. This tumor provides a fortunate situation for observations on the genetic relationship of primordial, vesicular and clear cells; all manner of transitional stages between these three cell types are present.

Apparently the histologic structural pattern of parathyroid adenoma is subject to considerable variation. Of these variations certainly the monotonous uniformity found in case 1 is the simplest. In the tumor of case 2 the colloid-filled follicles and extrafollicular droplets of colloid add variety to the structural arrangement; I am of the opinion that such

12. Cope, O.: Surgery **16**:273, 1944.
13. McQuillan, A. S.: Ann. Surg. **108**:464, 1938.

histologic variations are largely accidental and that no important significance can be attached to them. Similar findings are observed from time to time in normal glandules.

Case 3 illustrates a most unusual bit of parathyroid pathology. There is nearly a total infarction of the adenoma. The infarction is recent, and the histologic changes (necrosis and cellular reaction in the capsule of the tumor) are such in character and degree as would correspond to the patient's clinical history of twenty days' duration. From the specimen removed at operation it is not possible to determine the cause of the infarction. However, the clinical effect of the infarction was dramatic; it produced sudden severe hypoparathyroidism, associated with spasms and convulsions and with an alarming degree of hypocalcemia. Indeed, the spontaneous symptoms of hypoparathyroidism with which this patient suffered were similar to those of the hypoparathyroidism which may follow surgical removal of a parathyroid adenoma. This case is interesting also from the point of view of interpreting the function of the parenchymal cytologic elements of the gland. Although there were several thin layers of uninjured parenchyma in the capsule of the adenoma, and although these layers were made up of vesicular and clear cells, they were not functionally adequate to prevent the development of a hypoparathyroid state. One may conclude that at least within certain limits there is a quantitative relationship between the amount of parathyroid parenchyma and the secretory activity of the glandules, and the clinical state of the patient.

## SUMMARY

In 5 cases of parathyroid adenoma the tumor was single and of moderate size.

In 3 of the cases the adenoma was associated with hypercalcemia, hypophosphatemia and the skeletal changes of osteitis fibrosa cystica generalisata. In 2 of these 3 cases there were manifestations of renal calcifications and lithiasis.

In 1 case there was nearly complete infarction of the parathyroid adenoma. The patient came under clinical observation in a severe state of hypoparathyroidism (hypocalcemia and convulsions).

In 1 case the tumor was a true oxyphil adenoma. This is a rare neoplasm. It was associated with no skeletal or renal lesions.

Although more than 300 cases of adenoma of the parathyroid glands have been reported in the literature, knowledge of primary hyperparathyroidism is still incomplete.

Primary hyperparathyroidism provides a favorable situation for analyzing the function of the different kinds of parathyroid cells in an effort to correlate their histologic structure and chemical activity; such study promises an advance toward understanding the normal physiologic and metabolic functions of these cell types.

# FUNCTIONING OF THE FETAL KIDNEY AS REFLECTED BY STILLBORN INFANTS WITH HYDROURETER AND HYDRONEPHROSIS

L. J. WELLS, Ph.D.
AND
E. T. BELL, M.D.
MINNEAPOLIS

JUDGING from observations in fetal rats,[1] we believe that the rate of secretion of fetal urine is much more rapid than formerly supposed.[2] The rate may be accelerated experimentally by injecting urea under the skin of the fetus or by ligating the renal pedicles of the mother.[3]

A review of literature for evidence that the human kidney functions before birth led to the realization that there are surprisingly few records of stillborn infants who showed hydronephrosis or urine in the bladder or both. In fact, the only records found were those of 6 infants observed by Dohrn.[4j] The quantity of urine in the bladder ranged

From the Departments of Anatomy and Pathology, University of Minnesota.

1. Wells, L. J.: Anat. Rec. **94**:504, 1946.

2. (*a*) Gersh, I.: Contrib. Embryol. **26**:33, 1937. (*b*) Windle, W. F.: Physiology of the Fetus, Philadelphia, W. B. Saunders Company, 1940. (*c*) Preyer, W.: Specielle Physiologie des Embryo, Leipzig, Grieben, 1885.

3. Wells, L. J.: Proc. Soc. Exper. Biol. & Med. **62**:287, 1946.

4. (*a*) Hinman, F.: The Principles and Practice of Urology, Philadelphia, W. B. Saunders Company, 1935, vol. 1. (*b*) Parmalee, A. H.: The Newborn Child, in Curtis, A. H.: Obstetrics and Gynecology, Philadelphia, W. B. Saunders Company, 1933, vol. 1. (*c*) Bell, E. T.: A Textbook of Pathology, Philadelphia, Lea and Febiger, 1944. (*d*) Needham, J.: Chemical Embryology, London, Cambridge University Press, vol. 3, pp. 1255-2019, 1931, (*e*) Gruber, G. B.: Missbildungen der Harnorgane, in Schwalbe, E.: Die Morphologie der Missbildungen des Menschen und der Tiere, Jena, G. Fischer, 1927, pp. 157-374. (*f*) Ahlfeld, F.: Arch. f. Gynäk. **14**:276, 1879. (*g*) Virchow, R.: Gesammelte Abhandlungen zur wissenschaftlichen Medicin, Hamm, G. Grote, 1862. (*h*) von Bischoff, T. L. W.: Entwickelungsgeschichte der Saugethiere und des Menschen, Leipzig, Leopold Boss, 1842. (*i*) Englisch, J.: Arch. f. Kinderh. **2**:98, 1881. (*j*) Dohrn: Monatschr. f. Geburtskunde u. Frauenkrankheiten. **29**:105, 1867. (*k*) Cameron, G., and Chambers, R.: Am. J. Physiol. **123**:482, 1938. (*l*) Makepeace, A. W.; Fremont-Smith, F.; Dailey, M. E., and Carroll, M. P.: Surg., Gynec. & Obst. **53**:635, 1931. (*m*) Guthmann, H., and May, W.: Monatschr. f. Geburtsh. u. Gynäk. **91**:306, 1932. (*n*) Litzmann: Fötalleben, in Wagner, R.: Handwörterbuch der Physiologie, mit Rücksicht auf physiologische Pathologie, Braunschweig, F. Vieweg u. Sohn, 1842-1853, vol. 3, pt. 1, p. 91. (*o*) Hecker, C.: Virchows Arch. f. path. Anat. **11**:217, 1857. Gersh.[2a] Windle.[2b] Preyer.[2c]

from 4 to 105 cc., and only the infant having the most urine showed hydronephrosis (*Ureteren und Nierenbecken zu Cysten entartet* [ureters and renal pelves degenerated to cysts]).[5]

In searching the necropsy files of the department of pathology of the University of Minnesota for such records, we centered attention on those of the 52 stillborn infants which showed spina bifida. These infants would be expected to exhibit a high incidence of anomalies. The finding of a single one with dilatation of the urinary passages would constitute evidence that fluid had been secreted previous to the interruption of the placental circulation. Four were found (table).

Considering the anatomic causes of the dilatations and the steps in embryonic development leading to the anomalous conditions, we find it obvious that in the first infant the obstruction of the passages was due to the blind ending of the ureters associated with absence of

*Stillborn Infants with Hydroureter and Hydronephrosis*

| Infant | Sex | Crown-Heel Measurement, Cm. | Age, Lunar Months * | Anomalies of the Urinary Passages | | |
|---|---|---|---|---|---|---|
| | | | | Dilatations | Obstructions | Other Anomalies |
| 1 | F | .. | 7† | Bilateral hydroureter (diameter of ureters, 1 to 2 cm.) | Blind ending of ureters | Absence of bladder and urethra |
| 2 | F | 53 | 10 | Left hydroureter | Thin membrane closing left ureteral orifice | Horseshoe kidney |
| 3 | M | 36 | 7 | Bilateral hydronephrosis and hydroureter (slight) | None recorded | None recorded |
| 4 | M | 45 | 9 | Left hydronephrosis and hydroureter | None recorded | None recorded |

* The ages of the second, third and fourth infants were estimated by interpolation from Richard E. Scammon's table of ages based on crown-heel measurements (unpublished).
† The age of this infant was estimated from the menstrual history of the mother.

the bladder and urethra. The presence of ureters shows that early in development the wolffian ducts had entered the cloaca, since in embryos of chicks agenesis of the ureter may be produced experimentally by preventing the wolffian duct from reaching its normal destination.[6] The presence of kidneys indicates that the ureteric buds, acting as organizers, had caused the metanephric blastemas to differentiate into secretory tubules, because in human embryos nonunion of bud and blastema results in agenesis of the kidney.[7] Subsequent to these steps there had been arrested development of the cloacal region. This is

5. We did not attempt an exhaustive survey of the literature, and we excluded from consideration numerous records in which it was impossible to determine whether the infants were stillborn (Englisch [41]; Dohrn [41]; Makepeace and co-workers.[41]).

6. Boyden, E. A.: Anat. Rec. **52**:325, 1932.

7. Gruenwald, P.: Anat. Rec. **75**:237, 1939. Boyden.[6]

shown by the fact that at autopsy the infant lacked bladder, urethra, genitalia, anus and rectum.

In the second infant the anatomic cause of the dilatation was the thin membrane which obstructed the end of the left ureter. Undoubtedly this membrane represents anomalous persistence of the ureteral septum, described by Chwalla.[8] In normal embryos this septum completely seals off the end of the ureter for a considerable period. It appears at the stage of development in which the ureter has just established its direct opening into the urogenital sinus, and it usually persists until about the 24 mm. stage. Although in this infant there was no other anomaly which reflected a retardation of development subsequent to the 24 mm. stage, the presence of a horseshoe kidney pointed to such retardation previous to this stage. A causative factor in the development of horseshoe kidney is a delayed passing of the kidneys through the crotch of the umbilical vessels, and the critical moment for fusion of the kidneys is during or near the 10 mm. stage.[9]

In the third and fourth infants there may have been membranes closing or partially closing the ends of the ureters that were not observed at autopsy. That a defective nerve supply of the bladder did not cause the hydronephrosis, as it may in cases of anencephaly,[4c] is suggested by the fact that in the third infant the bladder was listed as normal and that in the fourth the dilatation was unilateral.

These observations confirm those of Dohrn,[4j] and they provide evidence, as convincing as any thus far recorded, that in the human fetus the kidney secretes fluid. Cameron and Chambers [4k] found that as early as three and one-half months after conception the fetal kidney is able to concentrate vital dyes. Also, analysis of the fluids of pregnancy suggests that fetal urine enters the amniotic sac, especially during the last half of gestation.[4l,m] Furthermore, in some cases of birth by breech presentation, it has been noted that previous to delivery of the head a small amount of liquid emerged from the urethral orifice.[10] Contrary to one point of view,[11] it would seem that the several observations constitute ample evidence for concluding that the human kidney functions during the prenatal period, especially when these observations are considered in the light of the rapid secretion of urine by the kidney of the fetal rat.[12]

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8. Chwalla, R.: Ztschr. f. Anat. u. Entwcklngsgesch. **83**:615, 1927.
9. Boyden, E. A.: Anat. Rec. **51**:187, 1931. Boyden.[6]
10. Litzmann.[4n] Hecker.[4o]
11. Gruenwald, P., and Popper, H.: J. Urol. **43**:452, 1940.
12. Footnote 3.

# EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS

## Changes Produced in Skeletal Muscle

RUDOLF ALTSCHUL, M.U.Dr.
SASKATOON, SASKATCHEWAN, CANADA

EXPERIMENTAL arteriosclerosis may or may not be identical with, or related to, human arteriosclerosis. Nevertheless, experimental arteriosclerosis is an excellent means of studying vascular changes and their sequelae and of revealing potential reactions of the vascular system.

The present report deals with experimental cholesterol arteriosclerosis. The effect of feeding egg yolk and other cholesterol-containing foods or pure cholesterol to herbivores has been amply studied. Extensive reviews have been contributed by Duff [1] and Hueper.[2]

The greater part of the arterial tree becomes involved, as well as many other tissues and organs. Yet, until recently it has seemed impossible to produce changes in vessels of the central nervous system, in peripheral nerves and in skeletal muscle. I [3] have succeeded in producing vascular changes in the cerebral vessels of 5 of 17 rabbits, in addition to the regular changes in the choroid plexus which have been described previously. In several rabbits the peripheral nerves were also affected. The skeletal muscle was examined in 17 rabbits, 7 guinea pigs and 2 golden hamsters, and in all of these animals with the exception of 2 guinea pigs which died shortly after the beginning of the experiments, muscular changes were evident. The description of the lesions occurring in skeletal muscle follows.

### METHODS

The animals were given a diet of milk, powdered yolk and yolk cake. The yolk cake was baked from a dough of 4 volumes (approximately) of yolk powder, 1 volume of flour, yeast, water and a little salt. To this diet some hay was added from time to time. A number of rabbits received, either from the beginning of the experiment or later on, a daily dose of 0.3 Gm. of pure cholesterol in capsules. No oil was given to facilitate the absorption of the cholesterol because the fat contained in the milk and in the yolk sufficed for this purpose.

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From the Department of Anatomy, University of Saskatchewan.

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1. Duff, G. L.: Arch. Path. **20**:81 and 259, 1935.

2. Hueper, W. C.: Arch. Path. **38**:161, 245 and 350, 1944; **39**:51, 117 and 187, 1945.

3. Altschul, R.: J. Neuropath. & Exper. Neurol., to be published.

The animals died or were killed at certain intervals. Skeletal muscle was excised from different regions, mainly from the thigh, the dorsal musculature, the diaphragm, the forelegs, the masseters and the tongue. It was fixed twenty-four to forty-eight hours in 4 per cent formaldehyde solution, then trimmed, dehydrated and embedded in paraffin. Sections were stained by the following methods: hematoxylin-eosin; cresyl violet; Mallory's for connective tissue; von Kossa's for calcium; Gömöri's for iron.

## RESULTS

The results varied greatly from one animal to another and among muscles of different regions. Two different types of lesions were observed, namely, those of the muscle fibers proper and those of the blood vessels in the interstitial tissue. In 1 case alterations were found inside a muscle spindle. Nerves of the muscles were also affected, but these lesions have been reported elsewhere.[3]

*Changes in the Muscle Fiber Proper.*—At least three types of changes were observed. The first and most severe was waxy (hyaline) degeneration of the sarcoplasm with disappearance of fibrillae and muscle nuclei. Frequently, this change affected only parts of the fiber. In these cases, the waxy part of the degenerated fiber was round and wider than normal, forming a kind of homogeneous eosinophilic mass intercalated in the fiber. When the adjacent portions of the fiber were affected, they were abnormally thin, were basophilic and showed nuclear proliferation.

In the second type of change the damage was less severe. Here, the sarcoplasm turned distinctly basophilic, lost its cross striation, became granular and broke into particles. The Kossa reaction for calcium salts was distinctly positive, while the iron reaction of Gömöri revealed only traces of iron. I could not determine whether the presence of calcium salts was responsible for the basophilia of the fibers or merely accompanied it. The nuclei of the muscle did not disappear. On the contrary, they proliferated to a lesser or greater degree. They were frequently surrounded by some basophilic substance and separated from one another. In this event they may be regarded as muscle corpuscles being liberated from the syncytium of a single muscle fiber.

The separation of the muscle corpuscles from the degenerating fiber corresponds possibly to what Pfuhl [4] has termed dissociating degeneration, though in his opinion it occurs only in the first thirty-six hours after acute injury of the muscle fibers, and all the liberated muscle corpuscles degenerate soon. Pfuhl's statement is based on experiments of short-lasting injury (trypan blue injected into and around skeletal muscle).

In some cases the proliferating nuclei accumulated in piles on the periphery of the fiber, abandoning, as it were, the degenerated sarcoplasm and fibrillae (fig. 1). This anuclear fiber material became still more basophilic and calcified.

4. Pfuhl, W.: Ztschr. f. mikr.-anat. Forsch. **41**:569, 1937.

In a few cases the proliferation of nuclei was extreme; hundreds of nuclei appeared in greatly enlarged fiber portions where before the onset of the change two or three nuclei may have occurred (fig. 2). Under such circumstances it was not surprising to see some degenerating nuclei, for, as Bloom [5] stated: ". . . in most rapidly growing tissues, there is an occasional degenerating cell." Moreover, in the present case one was dealing with a pathologic condition.

In the third type of change the damage was even less severe. The fiber was very thin, the sarcoplasm was basophilic and the nuclei, after migrating to the center of the fiber, proliferated and formed longitudinal nuclear rows. There were differences in degree, leading gradually to the normal fiber.

None of the changes thus far described are specific for experiments with cholesterol feeding. All of them may occur in traumatic injuries, some of them in nutritional myodegeneration (Goettsch and Pappenheimer [6]; Chor and Davenport [7]), in avitaminosis E (Pappenheimer [8]), in toxemias and infectious diseases (Zenker's well known waxy degeneration in typhoid), while the thinning of fibers with nuclear proliferation occurs regularly in denervation. Therefore, it is logical to attribute these changes not to arteriosclerosis or to cholesteremia directly but, until the contrary is shown, to the serious changes affecting the liver and to the lesser changes of the gastrointestinal tract, which are always present. Thus the damage of the muscle fibers herein described may be regarded as nutritional myodegeneration, leaving the interpretation of the term "nutritional" open to discussion.

A result difficult to explain is the spotty distribution of most of the changes occurring in my animals, which affected some fiber or a few fibers, while most of the surrounding fibers appeared normal (fig. 3). This irregularity may be attributed to the blood supply or to local vascular changes, but from the microscopic examination no conclusions could be drawn which would supply a satisfactory explanation.

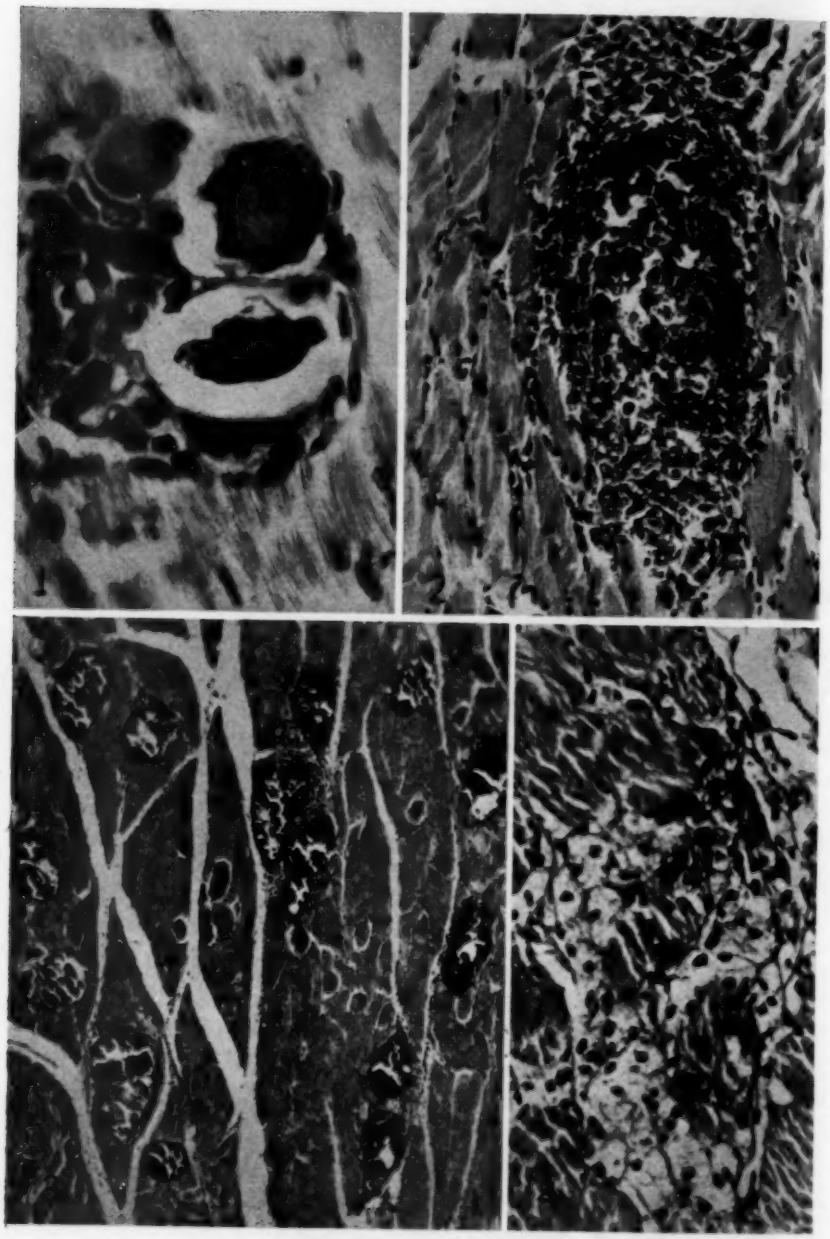
A change which has to be regarded as specific for cholesterol arteriosclerosis is the transformation of muscle corpuscles into foam cells. It remains to be shown whether the same pathologic condition may be brought about by other macromolecular substances, such as polyvinyl alcohol. Though this change was rather rare, it was distinct: In portions of degenerated skeletal muscle fibers the foam cells were crowded against each other and, in some cases at least, filled the sarcolemma completely (fig. 4). There may be a simple explanation of the origin

5. Bloom, W., in Maximow, A. A., and Bloom, W.: A Textbook of Histology, Philadelphia, W. B. Saunders Company, 1942, p. 227.

6. Goettsch, M., and Pappenheimer, A. M.: J. Exper. Med. **54**:145, 1931.

7. Chor, H., and Davenport, R. E.: Arch. Path. **27**:497, 1939.

8. Pappenheimer, A. M.: Am. J. Path. **15**:179, 1939.



FIGURES 1, 2, 3 AND 4

*(See legends on opposite page)*

of the foam cells: that they were immigrated histiocytes and were removing a degenerating fiber. But there was no trace of the original muscle nuclei, nor was there any sign of histiocytes that were in the stage of immigration. On the other hand, a gradual transformation of muscle corpuscles into foam cells was seen several times. Finally, foam cells have not been found in skeletal muscle under conditions other than experimental cholesterol arteriosclerosis. When one takes into account the fact that in the course of experimental arteriosclerosis different cell types may turn into foam cells (Hueper [2]; Altschul [3]), it appears likely that in the cases under consideration the muscle corpuscles which originated by dissociation of the damaged fiber became foamy. If that is the case, it remains to be determined whether these muscle corpuscles actively absorbed the fatty degenerated sarcoplasm or the lipoid material from the tissue fluids, or whether they were only fatty degenerated cells and not phagocytes.

The opinion that muscle corpuscles may act as histiocytes has previously been advanced (see review by Mayenburg [9]) but is not generally accepted. According to Pfuhl's experiments with intramuscular injections of trypan blue, muscle corpuscles are not phagocytic. But Forbus [10] reported distinct phagocytic activity on the part of these elements, a fact which I could confirm in hitherto unpublished experiments.

Besides the spotty manner in which the lesions were distributed inside the single muscle, there was some variability depending on the muscle region. The changes were found in muscles of the hindlegs, in the long dorsal muscles, in the diaphragm, in the muscles of the forelegs, in the muscle coat of the esophagus, in the masseters and in

9. Mayenburg, H. V.: Die quergestreifte Muskulatur, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol. 9, pt. 1.

10. Forbus, W. D.: Arch. Path. **2**:486, 1926.

### Explanation of Plate.

Fig. 1.—Tongue of a rabbit fed a milk and yolk diet ninety-five days (unretouched photomicrograph; hematoxylin-eosin; × 700). Note detachment and proliferation of muscle corpuscles. The sarcoplasm and the fibrillae are retracted and basophilic.

Fig. 2.—Masseter of a rabbit fed a milk and yolk diet one hundred and twenty days (unretouched photomicrograph; hematoxylin-eosin; × 200). A focus of nuclear proliferation is seen with degeneration of sarcoplasm and disappearance of fibrillae. There is little doubt that nearly all the nuclei are proliferated muscle nuclei and not, as may be assumed, nuclei of histiocytes.

Fig. 3.—Muscle of a foreleg of the animal whose masseter is shown in figure 2 (unretouched photomicrograph; hematoxylin-eosin; × 50). Note degeneration of the sarcoplasm, with basophilia, and pronounced nuclear proliferation.

Fig. 4.—Muscle of a foreleg of the animal whose masseter is shown in figure 2 (unretouched photomicrograph; hematoxylin-eosin; × 300). Foam cells are seen in muscle fibers.

the tongue. The strange fact was that, generally speaking, these lesions appeared earlier and were more pronounced in the muscles of the hindlegs and occurred later and were less accentuated or did not occur at all in the tongue and in the masseter muscles. The occurrence and the intensity of the changes in the other regions ranged between these two extremes. Goettsch and Pappenheimer [6] in their experiments on nutritional myodegeneration found the tongue and the masseter muscles completely normal. In other experiments Pappenheimer [8] found only the skeletal muscle of the tongue to be free of alterations.

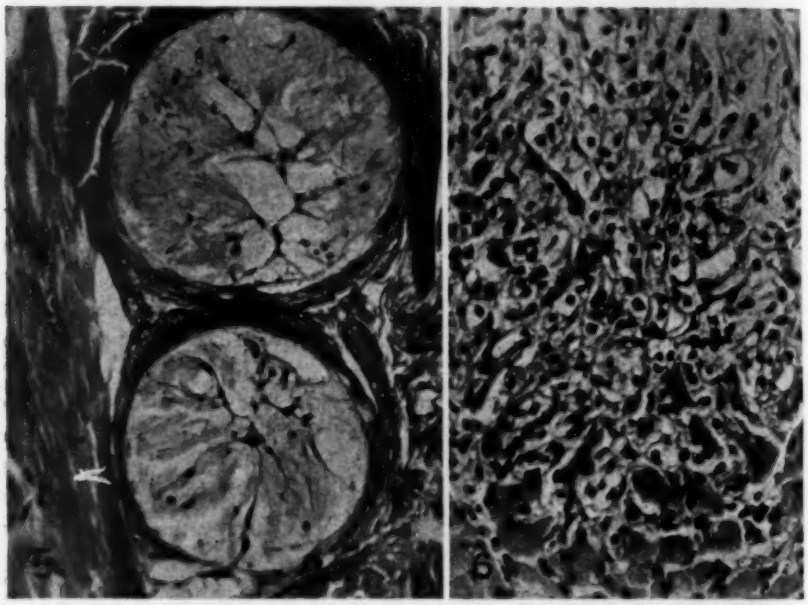


Fig. 5.—Tongue of a rabbit fed a milk and yolk diet one hundred and twenty-four days (unretouched photomicrograph; hematoxylin-eosin; × 175). Two vessels with "lipoid cushions" in the subendothelial layer are seen. Note that the lumens are only virtual.

Fig. 6.—Tongue of the same animal (unretouched photomicrograph; hematoxylin-eosin; × 250). Thinned muscle fibers are seen at the bottom and numerous foam cells in the center and at the top (foamy muscle corpuscles?).

In contrast, vascular changes were found much more frequently in the tongue and the masseter muscles and more rarely and to a less extent in the other muscle regions.

The vascular changes were seen mainly as deposits of lipoid material in the subendothelial spaces of the arterioles (fig. 5). The deposited material appeared as a light eosinophilic amorphous substance in the hematoxylin-eosin stain, while in Mallory's connective tissue stain the same substance was bluish. Its accumulation in the subendothelial space caused, in many instances, not only narrowing of the lumen but an

increase of the diameter of the vessel and thinning of the media. In contrast to the vascular changes in some other organs or tissues, the cellular reaction to the lipoid deposit was slight; only a few foam cells or spindle cells were found in the amorphous masses. The former may have been detached endothelial cells or immigrated histiocytes; the latter, metaplastic endothelial cells or fibroblasts from the media or the adventitia. Quasi-identical vessels were encountered in the heart and in the muscle wall of the stomach.

It is important to note that these vascular changes were not directly connected with the changes in the muscle fibers which have been described. Frequently no vascular changes were evident in muscles with parenchymal lesions, while vascular changes apparently caused no damage to muscle tissue proper. But in a few instances, though distinctly only in the tongue, areas were found in which the marginal zone showed a reduction in width of muscle fibers with no other parenchymal damage (fig. 6). In the center was an increase of cells, the majority being of the foam cell type. Whether they were immigrated or autochthonous histiocytes, fibroblasts of the endomysium or foamy transformed muscle corpuscles is open to discussion. The microscopic picture favors the last interpretation, although it does not exclude the possible participation of other cell types. As for the pathogenesis of these foci, I believe that they are caused by insufficiency of the blood supply, due probably to slow occlusion of a blood vessel.

It has already been mentioned that except in the choroid plexus no changes had previously been observed in the vessels of the central and the peripheral nervous system but that I [3] was able to provoke them in several animals. This, I suggested, might be due to the fact that in my experiments the yolk had been heated in baking the cake and its cholesterol thus rendered more toxic. This hypothesis, based on the difference in findings between my experiments and those of previous workers in which no heated cholesterol was used, is supported by the observation that gastric tumors may be produced by feeding heated cholesterol (Beck, Kirby and Peacock [11]). The lesions of skeletal muscle may have been brought about by this same increase of toxicity.

## SUMMARY

By feeding rabbits, guinea pigs and golden hamsters a diet rich in cholesterol, changes were provoked in skeletal muscle. They may be grouped into changes of the muscle fibers proper and changes of the blood vessels. In the first group there are varying degrees of intensity: (*a*) waxy degeneration, (*b*) granular necrosis with separation of the

11. Beck, S.; Kirby, A. H. M., and Peacock, P. R.: Cancer Research **5**:135, 1945.

proliferated nuclei and (*c*) thinning (atrophy) of the muscle fiber with basophilia and nuclear proliferation. These changes are not considered as specific for experimental cholesterol arteriosclerosis but rather as nutritional myodegeneration.

In several instances foam cells were found inside the sarcolemma, and it is suggested that they are foamy transformed muscle corpuscles.

The vascular lesion consists of a lipoid deposit in the subendothelium of the arteriole with a slight cellular reaction. The vascular lesions are not directly related to the intensity of the damage of the muscle fibers.

Heating of cholesterol may have some bearing on the intensity and the distribution of the lesions in experimental cholesterol arteriosclerosis.

# ATHEROSCLEROTIC VALVULAR DISEASE OF THE HEART

C. T. ASHWORTH, M.D.
DALLAS, TEXAS

SINCE 1904, when Mönckeberg [1] described calcific sclerosis of the aortic valve and attributed it to a degenerative process, numerous opinions have been expressed as to the causation of this lesion, some opposing and some supporting the original contention. Those opposing the view have usually stated the cause to be rheumatic fever.[2] Sohval and Gross [3] have reviewed the problem and have presented good evidence against rheumatic fever in some cases, subscribing to the atherosclerotic nature of the valvular disturbance.

The popular controversy as to the rheumatic or nonrheumatic nature of nodular calcific aortic valvular disease has diverted the attention of pathologists and clinicians from the manifest atherosclerotic changes of heart valves, although these changes are observed with remarkable frequency at autopsy. Most observers have directed their attention to the aortic valves,[4] although calcification of the mitral leaflets [5] and of the annulus fibrosus of the mitral valve [6] have been described occasionally. Hellwig [7] has recently given a rather careful description of the less severe form of atherosclerosis of the mitral valve, and Rytand and Lipsitch [8] have reported cases in which complete heart block and cardiac murmurs were associated with calcification of the mitral annulus fibrosus. Radiologists [9] have often demonstrated their ability to detect calcification in the cardiac valve areas, although their tendency has been to attribute most of it to old rheumatic valvular disease.

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From the departments of pathology of the Southwestern Medical College and Parkland Hospital.

1. Mönckeberg, J. G.: Virchows Arch. f. path. Anat. **176**:472, 1904.
2. Christian, H. A.: J. A. M. A. **97**:158, 1931. Karsner, H. T.: Proc. Inst. Med. Chicago **15**:62, 1944.
3. Sohval, A. R., and Gross, L.: Arch. Path. **22**:477, 1936.
4. Saltykow, S.: Beitr. z. path. Anat. u. z. allg. Path. **60**:321, 1915. Margolis, H. M.; Ziellesen, F. O., and Barnes, A. R.: Am. Heart J. **6**:349, 1930.
5. Sato, S.: Virchows Arch. f. path. Anat. **211**:238, 1911.
6. Giese, W.: Beitr. z. path. Anat. u. z. allg. Path. **89**:16, 1932.
7. Hellwig, C. A.: Am. Heart J. **24**:41, 1942.
8. Rytand, D. A., and Lipsitch, L. S.: Arch. Int. Med., to be published.
9. Sosman, M. C.: Am. J. Roentgenol. **50**:461, 1943.

It appears thus that the position of atherosclerosis in the production of changes in the cardiac valves is not fully appreciated. For this reason, a study of autopsy material and clinical records dealing with this problem has been undertaken.

## MATERIAL AND METHODS

The records of 500 autopsies were reviewed to determine the incidence of valvular lesions which were due to atherosclerosis. One hundred and one cases in which this type of lesion was demonstrable were found, and it is on these cases that this report is based. The heart was available for further gross study and special microscopic investigation in 63 of these cases.

Particular emphasis was placed on the study of the gross characteristics of the valvular lesions, the distribution of the lesions and the microscopic changes of early and late stages. Data derived from clinical and autopsy studies were arranged for the purpose of determining the relation between the valvular changes and cardiac failure, murmurs, blood pressure, age, sex, race, and atherosclerosis occurring elsewhere in the body.

Blocks for microscopic study were taken from the aortic and mitral valve areas and stained with hematoxylin and eosin and with sudan IV. The following blocks were taken [10]: one through the base of the aorta, the posterior aortic cusp, the intervalvular septum and the anterior mitral leaflet; one through the base of the aorta, the left aortic cusp and the left ventricle; one through the posterior mitral leaflet, the left auricular wall and the left ventricular wall; one transversely through the base of the aorta showing the commissure between the right and the posterior cusp. Other sections were taken as indicated by the nature of the gross lesions encountered.

## OBSERVATIONS

A high incidence of atherosclerotic changes involving the heart valves was encountered. The actual incidence cannot be determined in the 500 cases reviewed since slight atherosclerosis of the aortic and mitral valves was not always recorded in the protocols. However, in a group of 100 hearts which were reviewed grossly, the incidence of valvular atherosclerosis, including the earliest forms as well as the more advanced lesions, was 63 per cent. Of the total of 500 hearts, 36 (7.2 per cent) were found to have moderately advanced or marked atherosclerotic changes in the cardiac valves.

*Gross Appearance of Lesions.*—Usually the aortic and the mitral valve were both involved. In the earliest stages, however, the mitral valve was altered by slight atherosclerosis while the aortic cusps remained normal. The tricuspid and pulmonic valves were found to have escaped completely. The earliest lesion in the aortic valve was best seen from above. The aortic aspect of all cusps, usually approximately equally involved, was occupied by an opaque yellowish deposit extending onto the valve surface for one third to one half the distance of the valve

10. Gross, L.; Antopol, W., and Sacks, B.: Arch. Path. **10**:840, 1930.

and proximally across the sinus pocket to the region of the annulus of the aortic valve. Although some degree of atherosclerosis was almost always present in the first segment of the aorta, it was not usually continuous with that located in the valve itself. This yellow deposit often occurred without any grossly recognizable thickening or rigidity of the cusps, although such alterations were noted in some of the slightly more advanced lesions. The aortic surface only was involved in the process and usually, at this stage, the lipoid deposit could not be identified when viewed from the ventricular surface.

Commissural alterations were slight in almost all cases of atherosclerosis of the aortic valve. In about one half of the cases the commissures revealed no change whatsoever. In those instances in which calcium deposition and thickening of the cusps had taken place, the alterations were minor. The most common change consisted of slight agglutination of the lateral edges of two adjacent cusps. This fusion took place for a distance of about 1 mm. and was associated with slight yellow thickening of this portion of the cusps. In several cases the base of the aorta showed nodular plaques of atherosclerosis extending into the commissure, thickening and elevating it. Rarely, calcification was observed in these nodular plaques or in the commissure itself. In 8 of the 63 cases of atherosclerotic valvular disease available for gross study, small bridges of firm fibrous-like tissue were found somewhat below the commissural attachment, extending between two cusps. Such findings have been described by Sohval and Gross [8] as commissural bridges and are characteristic of atherosclerotic valvular disease, though they are seen in rheumatic aortic valvulitis. The so-called Lambl's excrescences [11] were observed on the aortic valves with frequency when atherosclerotic changes of the valve cusps existed.

In a great many cases the cusps of the aortic valve had undergone further alteration consisting of diffuse thickening or sclerosis. This thickening involved primarily the base of the cusps, but in several cases it extended to the free margin. Some degree of rigidity of the cusps was always present in these cases. Rolling of the free margin of the aortic cusps was not seen in any case of atherosclerotic valvular disease, although tension thickening of the margins was frequently observed. Plaques of calcific deposit occurred in most of these cases. They tended to be flat and were located in the basal portions of cusps, on the arterial surfaces. In more advanced calcific lesions there were roughened nodular masses up to 5 mm. in size, which projected into the sinus pocket. In 3 cases in which atherosclerotic thickening and fibrosis occurred there were found at autopsy small granular friable gray thrombi attached to the arterial surface, usually overlying an area of calcific deposit where

11. Gunzel: Beitr. z. path. Anat. u. z. allg. Path. **91**:305, 1933.

the endothelium had been ulcerated owing to the underlying plaque. These were devoid of any bacterial content. In no case was there actual shortening or contraction of the aortic cusps due to atherosclerotic changes.

In 5 cases the lesion of the aortic valve was extreme, leading to stenosis and also insufficiency. In these advanced lesions the calcific deposits were outstanding but still localized primarily to the aortic surface of the cusps, projecting into the sinus pocket region or onto the aortic surface of the cusp. The cusps were greatly thickened and rigid but not appreciably shortened. Commissural changes were impressive and consisted of extensive fusion of the lateral edges, which was associated with formation of large nodular plaques of calcium in the fused cusps. In these cases the left ventricular chamber was dilated and the wall markedly hypertrophied.

In cases of mild atherosclerotic valvular disease the intervalvular septum usually was the seat of focal rounded flat deposits of lipoid material. In cases of more advanced disease it had small to large calcific plaques in the subendocardial layers.

The anterior mitral leaflet was more intensely and more commonly involved than the posterior. Corresponding to the milder atherosclerosis of the aortic valve, in a great number of instances the mitral leaflets revealed multiple lipoid deposits on the ventricular surface. When the process was slightly more advanced, the leaflets were thickened, especially in their proximal portions, and rigid to some degree. Calcification occurred occasionally in the anterior mitral leaflet, rarely in the posterior, and not as frequently in either mitral leaflet as in the aortic cusps. In a few cases the leaflets of the mitral valve were rendered rigid and thickened by sclerosis and calcification to such a degree that the mitral orifice appeared to have been slightly stenotic and insufficient.

In 10 hearts the annulus fibrosus of the mitral valve was found to be occupied by a more or less complete ring of calcification. In these the mitral leaflets were also the seat of marked atherosclerosis with thickening and some degree of calcification. The calcific ring could be seen located in the base of attachment of the cusp, projecting somewhat inferiorly into the left ventricular wall and onto the ventricular surface of the posterior mitral leaflet. The calcific ring varied from 2 mm. to 6 mm. in diameter and was thickest about the midportion of the posterior leaflet, becoming smaller toward the anterior leaflet, and disappearing in the base of the anterior leaflet in some cases. The relationship of this calcification of the annulus of the mitral valve to certain clinical findings will be noted later. The distal portions of the mitral leaflets were comparatively thin in these hearts, and the chordae tendineae were thin, revealing no evidence of previous rheumatic fever. Furthermore, his-

tologic study of the valves and of the myocardium of these hearts excluded rheumatic fever as an etiologic factor.

*Microscopic Observations.*—The normal histologic structure of the aortic and mitral valves and the intervalvular septum [12] plays an important part in the development of atherosclerosis in these areas. The atherosclerotic changes regularly involve the surface of the aortic and mitral valves which are opposite to the outflow surfaces, that is, the arterial surfaces of the aortic valve and the ventricular surface of the mitral valve. The fibrosa and the adjacent fibroelastic coats of these surfaces, as well as the annulus fibrosus of the aortic and the mitral valve, are the sites of involvement almost to the complete exclusion of other layers or portions of the valves.

The annulus fibrosus of the normal aortic valve consists of a mass of dense, hyaline-appearing fibrous tissue extending a little above the valve ring and ending superiorly at the termination of the medial coat of the aorta. This annulus continues directly into the aortic cusp with the dense, hyaline fibrosa. Both of these structures, that is, the annulus and the fibrosa of the aortic cusp, are covered by the arterial fibroelastica. The layers of arterial fibroelastica, fibrosa, spongiosa and ventricular fibroelastica are well demarcated in the aortic valve. Beginning just below the ring spongiosa of the aortic valve is the dense fibrous layer, or fibrosa, of the intervalvular septum, also covered by a layer of fibroelastic tissue. The fibrosa of the septum is continued directly into the anterior leaflet, as the fibrosa of this cusp. Elsewhere in the mitral valve there is a distinct annulus quite similar in histologic structure to the annulus of the aortic valve.

It is apparent from the histologic study that the annulus fibrosus of the aortic and the mitral valve, the fibrosa of the two valves and the fibrosa of the septum decrease in cellularity with increasing age. The annulus sometimes assumes the histologic appearance of fibroelastic cartilage in the later age groups. Apart from the changes characteristic of atherosclerosis no apparent thickening or other abnormality can be identified as a part of the process of aging alone.

The earliest stage of atherosclerosis could be seen in the aortic or the mitral leaflets as an extracellular deposition of lipoid material. The deposition was so mild in some cases that on gross examination no evidence of it could be detected. In the area of the aortic valve the deposition of lipoid material occurred uniformly in the sinus pocket, involving mainly the fibroelastica and the adjacent portion of the annulus. The deposit extended onto the arterial surface of the cusp for a short distance, involving here the fibrosa and the arterial fibroelastica. The deposition was either confined to the fibroelastica or involved the fibro-

12. Gross, L., and Kugel, M. A.: Am. J. Path. **7**:445, 1931.

elastica and the superficial layers of the fibrosa. In the anterior mitral and to a lesser extent in the posterior mitral leaflet, early stages of involvement likewise showed histologic evidence that lipoid material was deposited in the outer portion of the fibrosa, and in the ventricular fibroelastic covering. In paraffin sections stained with hematoxylin and eosin these lipoid deposits were characterized by striking paleness, looseness and a foamy appearance. With fat stains, the loose spaces were found to contain large amounts of lipoid substance. Decreased cellularity of the involved coats was apparent in these cases. The lipoid deposit was often found composed of long needle-like crystalline formations resembling cholestrol crystals, arranged at right angles to the surface of the valve. In many cases there was a linear collection of macrophages, which were pale staining owing to contained cholesterol esters, along the arterial fibroelastica of the aortic valve and along the ventricular fibroelastica of the mitral.

These atheromatous lipoid deposits, in the early stage of the process, had produced slight thickening of the valve wall, but there was no other reaction of inflammation or vascularization. In more advanced lesions the lipoid deposit extended along the entire course of the leaflets and there was greater thickening of the valves. It was noted that even with advanced deposition of lipoid material there was no appreciable fibrous proliferation in any of the valve layers, although the fatty deposit itself had led to appreciable thickening and rigidity. In numerous instances scattered fine granular deposits of calcium salts were found in the aortic annulus, but massive calcification of this structure was never observed as it was with considerable frequency in the annulus of the mitral valve.

To be distinguished from these atheromatous lipoid deposits, there were in several cases formations of adipose tissue within the valves. In paraffin sections these were easily recognized by the presence of distinct fat cells with typical large signet ring arrangement. A similar appearance was noted also in the sudan IV stains, and in these the fat deposits took a deeper red stain than did the atheromatous deposits. Characteristic of this type of lipoid deposit of aortic and mitral leaflets was its occurrence without exception in the spongiosa. On some occasions this adipose tissue could be traced in the spongiosa through the valve ring into the adjacent pericardial wedge of fat. Adipose tissue formed in the heart valves, as described, was found with equal frequency in normal hearts and in those with valvular atherosclerosis, and is not considered, therefore, to be an abnormal finding.

A striking feature of severe forms of valvular atherosclerosis was deposition of large calcareous masses. The sites of predilection were in order of frequency: first the cusps of the aortic valve, distal to the sinus pocket, about the midportion or in the proximal half of the valve; second, the aortic sinus, overlying the annulus; third, the annulus fibro-

sus of the mitral valve; fourth, the annulus fibrosus of the aortic valve, and finally, the intervalvular septum and the proximal portion of the anterior mitral leaflet. These calcific masses thus appeared at the sites of previous lipoid deposits. The calcific deposits occurred on the ventricular surfaces of the mitral valve and the septum, and on the arterial surface of the aortic cusps, involving the fibrosa and the covering fibroelastica in these locations. On decalcification a homogeneous pink-staining acellular matrix remained. The other layers of the valve were essentially intact even in the presence of extensive valvular calcification. Around the borders of calcification there could usually be seen a slight zone of fibrosis and often a slight infiltration of lymphoid cells. In a few cases there was moderate vascularization of the fibrous zone surrounding the calcific deposits.

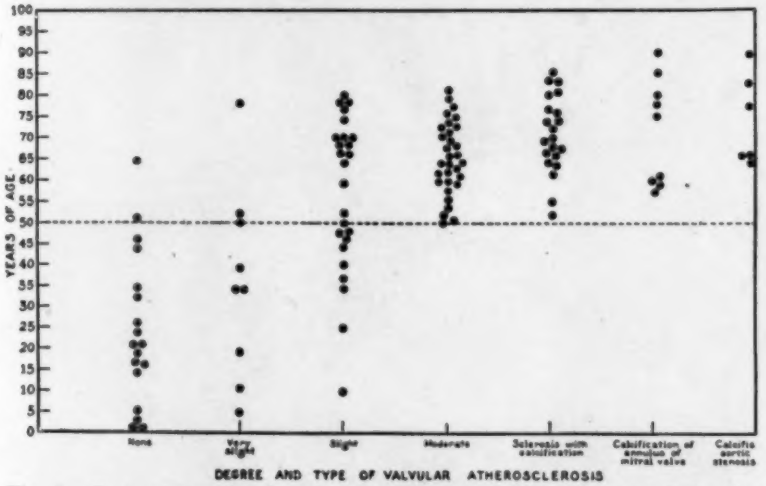


Fig. 1.—Scattergram showing the relationship of the incidence and the degree of valvular atherosclerosis to age.

## ACCESSORY CLINICAL AND PATHOLOGIC FINDINGS

*Age.*—It is quite evident that a correlation exists between the age of the patient and the occurrence of atherosclerosis of the aortic and mitral valves (fig. 1). In several patients below 10 years of age mild atherosclerosis of the anterior leaflet of the mitral valve was present. In these patients there was usually no accompanying atherosclerosis of the aortic valve or of the aorta. The same was true for most patients with atherosclerosis who were 25 years of age or less. In patients more than 25 years of age mild atherosclerosis of the aortic valve was often present in addition to that of the anterior mitral leaflet. The incidence of atherosclerosis increased markedly after 50 years of age, so that above this age atherosclerosis was almost always present to some degree in the valves.

Among 100 patients in whose case particular care was taken to detect the presence of mild atherosclerosis, there were 2 over 50 years of age in whom no atherosclerosis had occurred in the heart valves. There was no

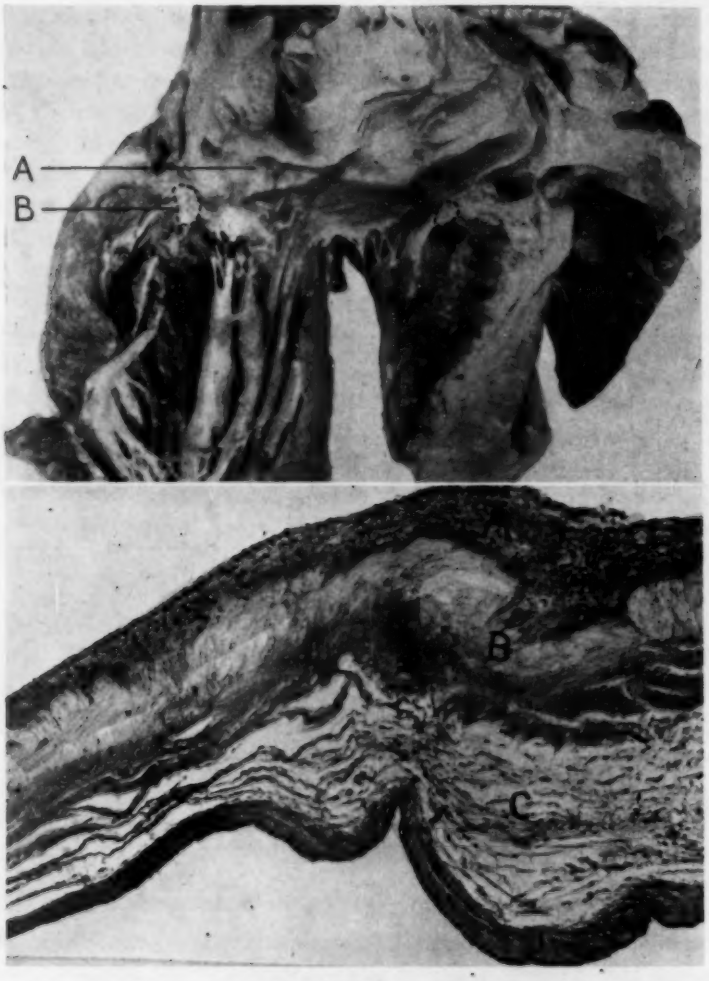


Fig. 2.—Upper part: Heart with calcification of the annulus fibrosus of the mitral valve. *A* is the contour of the ring as seen through the endocardium at the base of the posterior mitral leaflet. *B* is the cut section of the calcific ring.
Lower part: Aortic cusp with moderate atherosclerosis. Note in the arterial fibroelastica (*A*) the many macrophages containing lipoid material and observe the marked atherosclerosis of the superficial portion of the fibrosa (*B*). The spongiosa is entirely normal, as is the ventricular fibroelastica (*D*).

evidence of anything more than slight valvular atherosclerosis in patients under 50. The severity of the atherosclerosis seemed to increase with the age, so that about 42 per cent of patients over 50 were found to have

sclerosis and calcification, calcification of the mitral annulus or aortic stenosis.

*Race and Sex.*—The sex incidence in the 500 cases reviewed was 63.2 per cent males and 36.8 per cent females. The racial distribution was 53.2 per cent white persons other than Latin Americans, 40.4 Negroes and 6.4 per cent Latin Americans. Of the patients who had moderate or marked atherosclerotic valvular lesions, 77 per cent were males and 23 per cent females. Eighty and four-tenths per cent were members of the white race other than Latin Americans, 17.6 per cent were Negroes, and 2 per cent were Latin Americans.

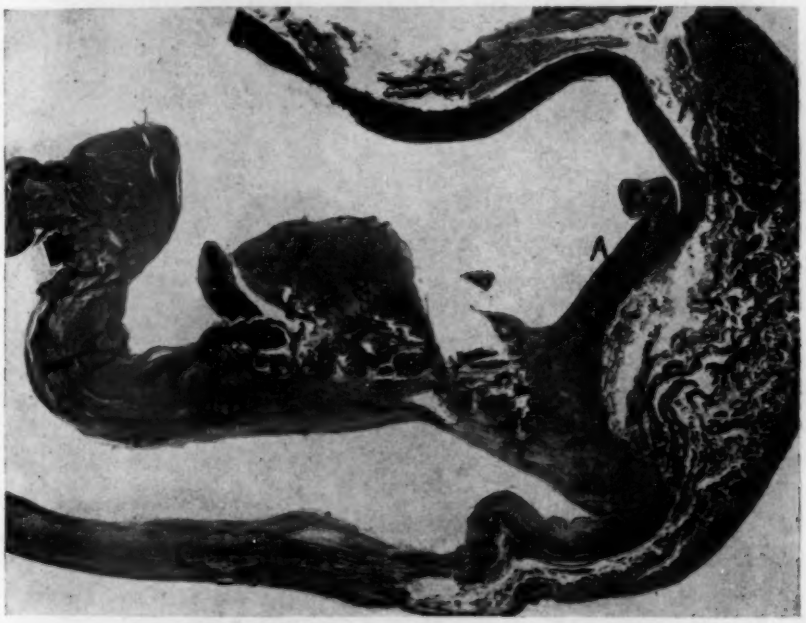

Fig. 3.—Photomicrograph of the base of the aorta (*A*), the posterior aortic cusp (*B*) and the anterior mitral leaflet (*C*). Marked calcific sclerosis of the aortic cusp and marked atherosclerotic thickening of the anterior mitral leaflet are shown.

*Relationship of Hypertension to Atherosclerosis of Cardiac Valves.*—The criterion for the existence of hypertension in the cases analyzed was systolic pressure above 150 mm. and diastolic pressure above 90 mm. of mercury or definite arteriolosclerosis of the kidneys. In the majority, both criteria were fulfilled. Of 101 patients with atherosclerosis of the heart valves, 39 had hypertensive disease. In the majority of cases this was classified as essential hypertension. Of 33 patients showing sclerosis with calcification, calcification of the mitral annulus and aortic stenosis, 16 were hypertensive and 17 nonhypertensive; of

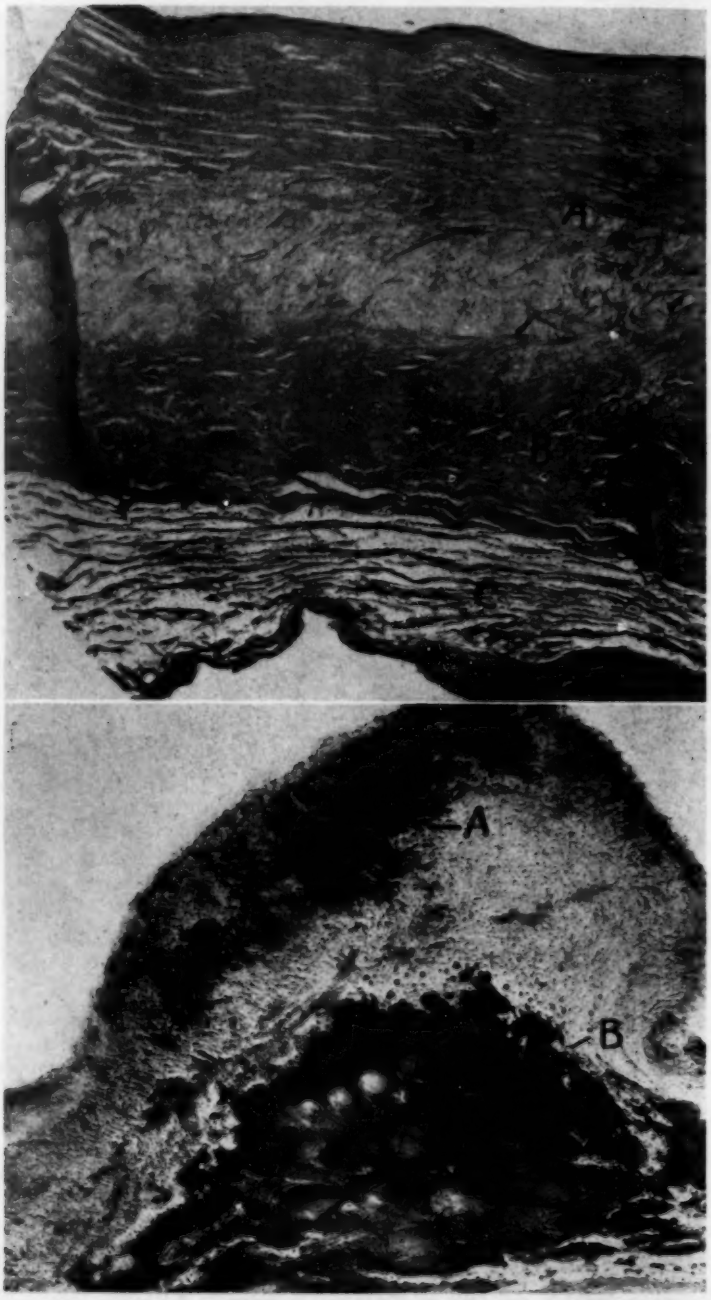


Fig. 4.—Upper part: Moderate atherosclerosis of the anterior mitral leaflet. Note the lipoid deposits in the fibroelastica and the superficial layers of the fibrosa (*A*). The remaining fibrosa shows a decrease in cellularity (*B*). The spongiosa (*C*) and the auricular fibroelastica (*D*) are normal.

Lower part: Fat stain of an aortic cusp showing lipoid deposits in the fibroelastica and the fibrosa as seen in atherosclerosis (*A*). Note that the adipose tissue extends into the spongiosa of the aortic cusp (*B*).

66 who had slight to moderate atherosclerosis, 23 were hypertensive and 43 were nonhypertensive. However, even though these observations might appear to indicate that there is some relation between more advanced valvular atherosclerosis and hypertension, the incidence of earlier ages and hence less hypertension, was considerably greater in those with only slight or moderate valvular atherosclerosis. Correcting for this disparity in age distribution by taking into consideration only patients 50 years of age or over, one found that of those with the more advanced forms of atherosclerosis, 16 were hypertensive and 17 nonhypertensive, as stated, while among the patients with the less severe forms there were 20 who were hypertensive as against 30 nonhypertensive. The difference in the incidence in the two groups is not apparently significant.

*Cardiac Murmurs in Cases of Valvular Atherosclerosis.*—In 21 of the group of 101 cases of valvular atherosclerosis murmurs were described in the clinical records. In the 5 cases in which there was aortic stenosis a systolic murmur was noted at the base of the aorta, while a diastolic murmur was found at the base in 3, a systolic murmur at the apex in 3 and a mitral diastolic murmur was noted in 1 case.

Of the 10 cases in which calcification of the annulus fibrosus of the mitral valve was found, murmurs were recorded in 5. In each of these 5 a systolic apical murmur, usually grade 1 or 2, was noted and in 1 there was a systolic and in 1 a diastolic murmur at the base. In each of the cases in which a systolic apical murmur was described, the mitral leaflets had been rendered rigid and thickened by sclerosis and calcification, in addition to the presence of calcification of the annulus. The ring of calcium was thicker in the cases with murmurs than in those without. In 2 of the cases in which calcification of the annulus fibrosus of the mitral valve was associated with murmurs there was complete heart block. This syndrome has been carefully described and discussed by Rytand and Lipsitch.[8]

Systolic murmurs of low intensity were described in 4 cases in which there was sclerosis with calcification of the mitral and aortic valves (exclusive of the cases in which there were calcification of the mitral annulus fibrosus and aortic stenosis). However, in 2 of these cases anemia of less than 10 Gm. of hemoglobin was present, leaving only 2 cases in which the murmurs might be attributed with some certainty to the valvular sclerosis and rigidity.

In the cases of slight or moderate atherosclerosis of the valves, 65 in number, murmurs were heard in 5. The murmur was mitral systolic in each instance. However, in each instance the murmur could be explained on some other basis than the valvular lesion: In 3 cases there was anemia of less than 10 Gm. of hemoglobin, in 1 case there was acute

bacterial endocarditis of the mitral valve and in 1 case there was old mitral valvulitis due to rheumatic fever.

*Relationship of Valvular Atherosclerosis to Atherosclerosis Elsewhere.*—The fact that the valvular atherosclerosis and the sclerosis occurring in arteries throughout the body were identical in nature suggests that some single underlying factor or group of factors might be at work in the production of these lesions and that a correlation between the atherosclerotic lesions occurring in the various locations could be established. Such is actually the case. As the atherosclerosis of the valves increases in severity, so also does the atherosclerosis of the first portion of the aorta and of the coronary arteries. It should be noted, however, that there is not necessarily, in fact not usually, any direct continuity between the aortic arteriosclerosis and the atherosclerosis of the aortic valve. In fact, in several instances aortic arteriosclerosis was absent or slight, while the valvular atherosclerosis was marked.

## COMMENT

Valvular atherosclerosis is a common lesion, although it does not often lead to the production of clinical manifestations. In 7 of 500 cases with autopsy, however, death was actually due to some form of atherosclerotic valvular disease. In 5 of the 7 cases calcific aortic stenosis was present; in 2 there was complete heart block due to calcification of the annulus fibrosus of the mitral valve. In several other cases cardiac murmurs without impairment of cardiac function were produced. Although minor embolic phenomena were not observed in any of the cases of this series, they might be expected to accompany atherosclerotic valvular disease, since thrombi were observed on the altered valves in a few cases. I have never observed subacute bacterial endocarditis occurring on an atherosclerotic valve, nor do I know of any recorded instance of it.

The most common valvular atherosclerosis is that of simple deposition of lipoid material. Sclerosis with thickening and calcification of the valves occurred in an appreciable but smaller number of the cases. Calcific aortic stenosis comprised a small group, but one of the utmost clinical importance, while calcification of the mitral annulus fibrosus was present in 10 cases. In 2 cases of the latter group complete heart block was produced. It is proposed that these various types of valvular lesions be referred to as forms of atherosclerotic valvular disease.

As to the genesis of atherosclerosis of the heart valves, the same general factors which enter into the development of arteriosclerosis elsewhere seem to be of importance.[13] It has been noted that atherosclerosis

13. Leary, T.: Arch. Path. **23**:185, 1943.

of the valves is closely related to age. Hypertension, if it has any effect at all, simply increases the severity of the lesion, and certainly is not the primary condition. The fact that the atherosclerosis is characteristically distributed with great constancy in the annulus fibrosus and in the fibrosa of the mitral and aortic valves would seem to be related in some way to causal or contributing influences, It has been noted that these regions of involvement in the connective tissue, which, even in the young person is relatively acellular, dense and devoid of blood vessels, decreases in cellularity with age. Certainly such an alteration in cellularity and in metabolic activity would be expected to predispose to deposition of calcium and perhaps also of lipoid material. In other regions of the body it is not unusual to find lipoid material deposited in the substance of dense hyaline fibrous tissues, as in connection with old productive inflammation in some instances, or in the common hyaline plaques which occur in the splenic capsule, or in the dense scarlike tissue which makes up the wall of an old hydrocele sac. One might expect, therefore, a similar deposition of lipoid material to take place in the dense hyaline tissue of the annulus fibrosus of the heart valves and in the fibrosa of the cusps. This deposition would further be expected to vary in intensity with the amount of cholesterol or other lipoid substances circulating in the blood, as it appears to do in arteriosclerosis.[14]

The distribution of the atheromatous deposits described suggests that tension and vibration might be factors concerned in the genesis of atherosclerosis of the valves. The aortic surface of the aortic cusps and the ventricular aspect of the mitral leaflets are the surfaces which are subjected to the greatest pressure and on which the greatest vibrating effect of the moving columns of blood would be exerted.

It appears that sex and race are factors of minor importance in the occurrence of atherosclerosis of the cardiac valves. There is some suggestion in the material presented that there is a slightly greater incidence in males than in females and that the disease occurs with greater frequency in the white race than in the Negro. Although Martens [15] and also Rytand and Lipsitch [8] reported calcification of the annulus fibrosus of the mitral valve to be more common in women than in men, in the 10 cases reported here, males predominated 6 to 4.

The similarity of the problem of valvular atherosclerosis to that of generalized arteriosclerosis is further suggested by the correlation shown to exist between the occurrence of aortic and coronary arteriosclerosis and that of valvular atherosclerosis.

14. Hirsch, E. F., and Weinhouse, S.: Physiol. Rev. **23**:185, 1943.

15. Martens, G.: Beitr. z. path. Anat. u. z. allg. Path. **90**:497, 1932.

SUMMARY

Atherosclerotic valvular disease occurs with considerable frequency and occasionally gives rise to significant clinical findings. These consist of aortic stenosis, heart block from calcification of the annulus fibrosus of the mitral valve, and precordial murmurs in some cases of sclerosis with calcification of the aortic and mitral leaflets. Mild and moderate valvular atherosclerosis without associated clinical manifestations is exceedingly common after 50 years of age. Hypertension was present in the cases of more intense valvular atherosclerosis slightly more frequently than in those in which the lesions were mild.

On gross examination atherosclerosis of the mitral and aortic valves was characterized by involvement of the aortic sinus pocket and the arterial surface of the adjacent proximal segment of the valve cusp, the intervalvular septum and the ventricular surface of the proximal portion of the mitral leaflets, particularly the anterior. An opaque yellow deposit was characteristic of earlier stages, followed in some cases by thickening and rigidity of the leaflets, calcification, stenosis and the formation of a calcific ring around the mitral valve. Microscopically, the earliest change was the deposition of lipoid material occurring in the fibroelastic coverings of the mitral and aortic leaflets, in the adjacent fibrosa and in the annulus fibrosus of the aortic and mitral valves. Slight calcific deposits were seen in the annulus of the aortic valve as was calcification in the regions of the lipoid deposits. Fibrosis and slight chronic inflammation were noted around the areas of calcific deposits. The valvular layers remained fairly well intact even in the presence of extensive calcific deposits. Macrophages containing cholesterol esters were found near the deposits of cholesterol in the heart valves.

Factors considered to be related to the development of atherosclerosis of the heart valves were: age, hypertension, the physiologic decrease in cellularity of the annulus and of the fibrosa of the aortic and mitral valves and the effect of tension and vibration on certain portions of the valves.

# REACTION OF THE RETICULOENDOTHELIAL CELLS TO SUBCUTANEOUS INJECTIONS OF CHOLESTEROL

**Experimental Animals: Mice**

EDNA H. TOMPKINS, M.D.
NEW HAVEN, CONN.

A SERIES of studies has been carried out in recent years with the aim of obtaining information concerning the responses of tissues, particularly those of the reticuloendothelial system, to specific lipids by correlation of histochemical technics. The subcutaneous area was chosen as the preliminary site of investigation with each lipid, before proceeding to the more complicated reactions of the system as a whole, because of the relative freedom from rapid admixture with other lipids which may be maintained in that locality and because of the relative simplicity of normal subcutaneous tissue and, therefore, the ease with which infiltrations and reactions of cells can be followed in that region and applied to the interpretation of events in more complex regions.

The studies to date have included the reactions to various classes of lipids which do not contain cholesterol. The reactions to synthetic triglycerides,[2] to individual phospholipids [3a] and to galactolipids [3] have been presented. Except in the case of the galactolipids, the reactions were found to involve chiefly the cells of the reticuloendothelial system, and the morphologic aspect of these cells varied characteristically with each class of lipids and, at times, even with the individual members of a class. Investigation of the reactions to the groups which contain cholesterol, i. e., cholesterol and its esters, with fatty acids, now seems indicated. The present study concerns the reactions to cholesterol.

## MATERIAL AND METHODS

Chemically pure cholesterol (Coleman & Bell) was used for these studies. It was kept as a stock solution in alcohol (0.10 Gm. to 100 cc. of 95 per cent alcohol) and was injected as a colloidal suspension in a 5 per cent dextrose solution. Fresh suspensions were prepared for each injection by slowly dropping the

From the Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tenn., and the Laboratory of Applied Physiology, Yale University, New Haven, Conn.

1. Footnote deleted by the editor.

2. Gray, M. E.: Am. J. Anat. **67**:361, 1940.

3. Tompkins, E. H.: (*a*) Bull. Johns Hopkins Hosp. **70**:55, 1942; (*b*) South. M. J. **33**:154, 1940.

4. Footnote deleted by the editor.

desired aliquot of stock solution into the desired amount of dextrose solution over a boiling water bath. The dextrose solution was stirred constantly as the solution of cholesterol was added, and agitation was continued until all odor of alcohol had disppeared. Boiling distilled water was then added to bring the volume of dextrose solution back to the original, and the emulsion was drawn into a tuberculin syringe and allowed to cool. For experiments to be terminated within three days after the injections the concentration of cholesterol in colloidal suspension was 0.05 per cent; for experiments to be terminated between three and four days, 0.10 per cent; for experiments to be terminated between five and twelve days, 0.15 per cent, and for experiments to be terminated later than fourteen days after the injections, 0.20 to 0.25 per cent. The 0.05 and 0.10 per cent emulsions were clear; the more concentrated ones, slightly opalescent. Suspensions with cholesterol in 0.05 and 0.10 per cent concentrations could be maintained in the syringe indefinitely. Under the microscope hanging drop preparations of the suspensions presented separate pinpoint specks, like chylomicrons, in brownian movement and without aggregates. These lent a dull glow to the drop in polarized light but did not appear as sharply defined globules or crystals. After an hour or longer the specks became converted into small aggregations of extremely fine needle-like crystals, which were sometimes definitively anisotropic.

The injections were made subcutaneously in the lower abdominal and lumbar areas of mice of the Wistar strain that had long been interbred in the laboratory. Adults of both sexes were used (the females nonpregnant). One-half cubic centimeter of the suspension was given in each injection. No evidence of irritation was observed. The two areas of any given mouse were injected at different intervals, which were so spaced that the tissue reactions of the same mouse would be representative of alternate periods of examination. Examinations were made at hourly intervals up to twelve hours after the injection, then every two hours up to twenty-four hours, every twelve hours up to seventy-two hours, every twenty-four hours up to twelve days and finally, every forty-eight hours up to twenty-one days after the injection.

The mice were kept under the routine laboratory conditions (Purina Chow,[5] oats, lettuce and water ad libitum) until such time as examination was desired. At that time the experiment was conducted essentially in the manner described by Gray [2] after injections of triglycerides. An anesthetic dose (5 mg.) of pentobarbital sodium was given intraperitoneally in 1 cc. of saline solution. When anesthetized, the mouse was exsanguinated by sterile cardiac puncture. The blood serum was obtained for use in succeeding experiments. The areas of injection were incised, and the skin was pushed back by blunt dissection. Tiny snips of connective tissue from the underlying areas were filmed on cover slips by

5. The Ralston Purina Company states that the ingredients of the chow are: meat meal, dried skim milk, riboflavin, carotene, cod liver oil, brewers' yeast, wheat germ, corn grit, wheat cereal, corn cereal, dried beet pulp, molasses, steamed bone meal and iodized salt. The chemical analysis shows:

| | Crude, % | Digestible, % |
|---|---|---|
| Protein | 23.0 | 19.0 |
| Fat | 5.0 | 4.7 |
| Fiber | 4.0 | |
| Ash | 7.0 | |
| Nitrogen-free extract | 54.0 | 48.0 |
| Moisture | 7.0 | |
| | 100.0 | 71.7 |

the aid of teasers. A few of these were immediately moistened with serum from a different mouse, superimposed on slides, rimmed with petrolatum and left in the incubator at 37 C. until stained supravitally. The serum was stained with neutral red just before use by adding 0.5 cc. to a tube in which 0.04 cc. of a saturated solution of neutral red in absolute alcohol had been previously evaporated to dryness. The other films were air dried and boxed for staining without further fixation.

The supravitally prepared films were counted differentially at 37 C. and were later examined under polarized light at room temperature. The following features were recorded at the time of these counts: the presence, the site and the character of crystals of cholesterol; the presence, the relative refractivity, the size and the cytoplasmic location of droplets of lipid; the presence of intracellular debris; the color, the refractivity, the size, the shape and the cytoplasmic location of the droplets stained with neutral red.

The fixed films were exposed to 1 per cent nile blue sulfate in water; to sudan IV (in 70 per cent alcohol at 37 C. according to the technic described in Lee's "The Microtomist's Vade Mecum" [6a]); to osmic acid gas by suspension of wetted films over a 1 per cent solution of osmic acid in water; to the Schultz technic for cholesterol as described by Lee [6b] and to digitonin with examination by polarized light for differentiation of cholesterol from its esters, according to the technic of Leulier and Revol.[7] The examination of these films was made first from mounts in glycerin jelly. The jelly was then removed with water, and control observations were made after mounting in balsam in order to determine, by virtue of the xylene present, whether failure to stain with a specific stain indicated merely absence of lipid or the character of the lipid present. The films treated with digitonin were later treated with ether in order to differentiate between the anisotropism due to the digitonin complex with cholesterol and that due to the presence of cholesterol esters.

The films stained with sudan IV were counterstained with Ehrlich's hematoxylin, and those exposed to osmic gas, with carmine. These films plus those stained with nile blue sulfate afforded satisfactory observation of nuclear characteristics for correlation with the various cytoplasmic observations.

A Bausch and Lomb polarizing attachment was used for all studies with polarized light. This was used in conjunction with a monocular microscope equipped with a fluorite oil immersion lens.

As the reaction to the injections progressed, it was found that the most comprehensive understanding of the sequence of events was to be obtained at the periphery of masses of cells rather than within such masses. The early reactions were diffuse and generalized, and scattered foci of crowed cells began to develop only after the reactions had been under way for some time. These foci comprised mixtures of primary and secondary reactions; the secondary reactions included degeneration of cells in the packed centers, appearance of autolytic products of degeneration, new reactions to depositions from degenerate cells, continuous filling of cells with cholesterol esters due to continuous exposure to organized crystals of cholesterol and increase of webs of fibrous tissue. The foci, therefore, did not present an uncomplicated sequence of events in the reactions to colloidal suspensions of cholesterol alone. The peripheries, on the other hand, presented a changing array of reactions that seemed to follow one another in orderly, uniform

6. Lee, A. B.: The Microtomist's Vade-Mecum, ed. 10, Philadelphia, P. Blakiston's Son & Co., 1937, (*a*) p. 283 and (*b*) p. 284.

7. Leulier, A., and Revol, L.: Bull. d'histol. appliq. à la physiol. **7**:241, 1930.

sequence. Once foci developed, therefore, most of the observations and all of the differential counts were made along several axes from a focus.

Controls consisted of films from equivalent areas of untreated mice and from mice that had been given at various intervals injections of the menstruum used for the experimental injections, i. e., the 5 per cent dextrose solution. The control films were stained, examined and counted in the different ways employed for the experimental films.

## EXPERIMENTAL DATA

The terms "tissue clasmatocytes" and "macrophages" are employed in the sense in which they were used by Gray.[2] Briefly, the term "tissue clasmatocytes" refers to the large phagocytic mononuclear cells which are normally present in connective tissues, and the term "macrophages" refers to the phagocytic mononuclear cells which develop from smaller cells that enter from the capillaries under sufficient stimulus. Other than these two, it is believed that all other cytologic terms to be employed are uniformly understood, although the cells themselves cannot be uniformly differentiated by different technics.

While differential counts were made from both the living and the fixed films of all experiments, these data have been omitted in favor of summarized statements concerning only key points in the experiments. The counts served, however, to indicate the times at which characteristic modifications occurred most frequently in the areas of reaction, and the necessity for accurate differentiation in the course of the counts required careful analysis of the morphologic character of every cell in the field.

Normal connective tissues in the areas under study contain few types of cells, and they are almost uniform from the point of view of morphology. Mast cells, fibroblasts and tissue clasmatocytes are the constant inhabitants; neutrophils and lymphocytes occur infrequently, and clumps of eosinophils at times. The tissue is like that found by Gray [2] in the guinea pig except that mast cells occur more frequently and the ratio of fibroblasts to clasmatocytes is higher (average 4.0).

The clasmatocytes of normal tissue are usually elongated and present in scattered groups. They contain various-shaped, medium-sized deposits of neutral red with a neutral reaction. The fibroblasts contain scattered, extremely fine drops of neutral red. Neither cell contains refractive drops of fat in living preparations and neither is anisotropic. The clasmatocytes are finely stippled with red after exposure to sudan IV but contain no larger deposits and do not react to the other stains for lipids. The fibroblasts do not react to any of the stains for lipids.

An attempt has been made to simplify the description of the reactions of the tissue clasmatocytes and macrophages observed after the injections of suspensions of cholesterol by presenting a schematic diagram in figure 3. The periods of time stated in the diagram are didactic and represent merely the intervals after injection at which the given morphologic changes occurred most frequently. It must be remem-

bered, however, that any given morphologic change occurred and regressed gradually and that cells in other stages of metamorphosis were always present. The diagram indicates merely the period at which cells with a given morphologic character occurred in greatest proportion.

All statements of time have been made to refer uniformly to the interval extending from "the time of injection." Repetition of this phrase is therefore omitted in those places where it would otherwise be desirable for the purpose of scientific certainty.

The areas of injection were edematous for about three hours, and both fibroblasts and tissue clasmatocytes contained large light-colored "fluid vacuoles" in addition to the usual denser deposits of neutral red. The latter were somewhat less sharp than normal. The edematous background glowed diffusely under polarized light, but otherwise there was no evidence of the injected cholesterol. Cholesterol was at times found to be aggregated on clumps of fibrin in films stained with digitonin, but usually it was so scattered and in such minute droplets as to fail detection.

The edema had subsided by the end of three hours, and infinitely fine acicular crystals were found scattered separately throughout the field. Great numbers of these adhered electively to the surface of the tissue clasmatocytes, but at no time did they adhere to mast cells or fibroblasts. The field remained diffusely anisotropic under polarized light up to twelve hours. The crystals were apparently too delicate to be seen as discrete anisotropic particles in that light. By twelve hours they began to show as discrete anisotropic rods (fig. 1, *A* and *B*).

Neutrophils began to enter from the vessels by the end of three hours, and the tissue rapidly became filled with them. They wove in and out between the free acicular crystals of cholesterol, but at no time did they acquire crystals. They began to degenerate and to acquire drops of sudanophilic fat at nine hours. They were never anisotropic and did not react to the other lipid stains. Most of them were degenerating at eighteen hours, and all had disappeared within sixty hours. They were somewhat less plentiful in the areas injected with dextrose solution, but otherwise the reaction was similar. Their presence in the experimental areas was obviously not directly concerned with the absorption and removal of the cholesterol except so far as the fat liberated by their death may have been of significance as a source of fatty acids.

During this period the fibroblasts exhibited many medium-sized deposits of neutral red and at times "fluid vacuoles" (fig. 1, *B*). In the supravital preparations they also contained many tiny isotropic drops of fat (fig. 1, *C*), which at times stained with sudan. They reacted similarly, but to lesser degree, after injections of dextrose solution. They were never found to contain cholesterol. Their content of neutral red was relatively normal again at sixty hours, but they continued to

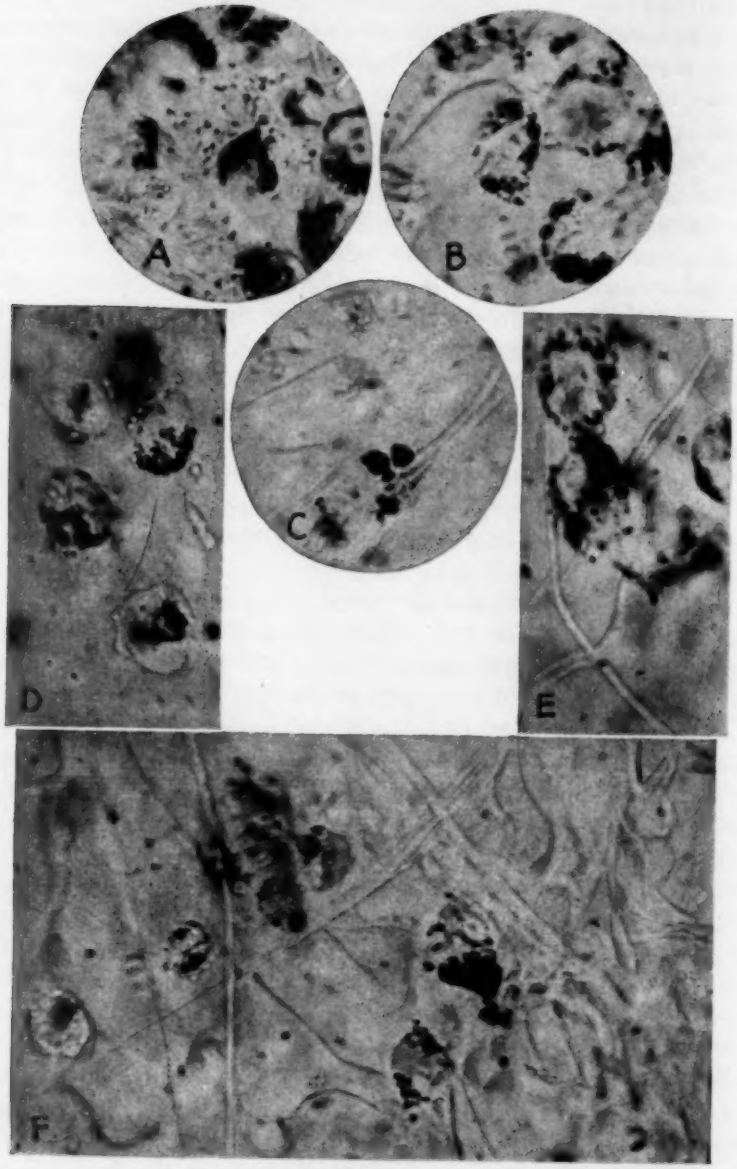


**Figure 1**

*(See legend on opposite page)*

have fine droplets of fat for eight to ten days. They often multiplied into dense capsules about foci of packed cells from that time on; since the initial reactions had subsided, this response seems secondary to autolysis and deposition of crystalline material within the foci, and not a part of the primary reaction to a colloidal suspension of cholesterol per se.

Mast cells took no part in the reaction. Eosinophils appeared in great numbers in some of the experiments but were so lacking in others that correlation to the injections of cholesterol seems unjustified, despite the frequent association of cholesterol and eosinophils in clinical material.

Up to twelve hours, then, the only cell that was specifically concerned with the cholesterol was the tissue clasmatocyte, and it was literally peppered with discrete acicular crystals. Neutrophils entered the area at three hours, but at no time concerned themselves with the cholesterol. Monocytes began to enter the area at nine hours. They were small and very motile and contained medium amounts of neutral red (fig. 1, *F*). They, too, wandered among the crystals of cholesterol without attracting them. They exhibited rapid increase in their own

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EXPLANATION OF FIGURE 1.

Photomicrographs of supravitally stained films of connective tissue from mice into which colloidal suspensions of cholesterol had been injected subcutaneously at various periods preceding the examinations. Deposits stained with neutral red appear gray or black, the depth being dependent on the intensity with which they stained. Drops of fat appear as rimmed white circles. Crystals appear as tiny dark or light specks or quadrangles, their color and shape being dependent on the focus at which they were viewed. × 781.

*A*. Mouse 94. Abdomen. Cholesterol in colloidal suspension was injected eighteen hours previously. Acicular crystals of cholesterol can be seen scattered throughout the area. Many adhere to the surfaces of the macrophages which had entered the field. The macrophages were small and easily distinguished from rounded tissue clasmatocytes at this stage.

*B*. Same as *A*. Two tissue clasmatocytes may be seen. Acicular crystals of cholesterol are adherent to the upper one. Both had acquired large, dark deposits of neutral red and a few vacuoles of isotropic fat. Fibroblasts are shown in the background. They contained considerable amounts of neutral red and droplets of isotropic fat but were devoid of crystals of cholesterol.

*C*. Mouse 94. Back. A colloidal suspension of cholesterol was injected twenty-four hours previously. A tissue clasmatocyte and a fibroblast are shown. The deposits of neutral red in the former were large and varied in color. Droplets of anisotropic fat were scattered at random in the cytoplasm. The fibroblast, at the top, contained isotropic fat only.

*D*. Same as *C*. Monocytes are seen developing into macrophages. They have scattered acicular crystals on the surfaces and a few drops of isotropic fat in the cytoplasm.

*E*. Two tissue clasmatocytes, which are in the same field as *D*, are shown. They contained considerable neutral red and vacuoles of anisotropic fat and were rounding. A few crystals adhered to the surfaces.

*F*. Same as *C*. A cluster of monocytes which are younger than those shown in *D*; crystals rarely adhered to the surfaces of such monocytes. The macrophage and the tissue clasmatocyte in the same field are similar to those illustrated in *A* and *B*, respectively.

size and in the number and the size of the deposits of neutral red (fig. 1, *D*). By eleven hours many contained scattered large drops of isotropic fat and by twenty hours had developed into macrophages (fig. 1, *A* and *F*). From then on they began to be as heavily coated with discrete fine acicular crystals of cholesterol as were the tissue clasmatocytes.

In the meantime, from about three hours after the injection the size and the number of neutral red deposits in the clasmatocytes increased rapidly until deposits of all sizes and shades of red filled the cells (fig. 1, *B, C, E* and *F;* also fig. 3, *1°* to *22°*). They soon became much darker, i. e., more acid in reaction, and more refractive. The cells often rounded and became apparently free. By six hours they also had large refractive drops of isotropic fat scattered at random among the deposits of neutral red, and often cellular debris. The crystals adherent to their surfaces in great numbers broadened slightly and often became alined somewhat in parallel, so that two or three lay side by side. By fourteen hours the crystals were definitely broader and thicker and often appeared as well defined anisotropic rods. By this stage many of the drops of lipid within the cytoplasm had become anisotropic. By twenty-two hours the crystals on the periphery of the clasmatocytes had so thickened that they had the shape of tiny grains of rice, and all of the isotropic drops in the supravital preparations had become replaced by anisotropic drops and exhibited the cross of polarization indicative of fluid crystals. These drops had also acquired a waxy consistency and appeared less refractive than the earlier isotropic drops. At this stage they stained faintly with sudan IV and osmic acid.

While these changes were occurring in the rounded clasmatocytes, the macrophages that were continuously developing from monocytes assumed more and more the same characteristics. Once they acquired the crystals of cholesterol on their surfaces, they pursued a course similar to that described for the tissue clasmatocytes, and by thirty-six hours after the time of the injection it became increasingly difficult to differentiate macrophages that had rounded from preexisting clasmatocytes and macrophages that had arisen by metamorphosis of infiltrated monocytes. By that time all crystals had disappeared from both types of macrophages and from the field; both types of macrophages contained debris and large refractive drops of lipid which did not stain supravitally, which now tended to be peripherally located, which contained beautiful crosses of polarization and which stained fairly deeply with sudan IV and faintly with osmic acid; both types of macrophages were swollen with a tremendous number of vacuoles of neutral red, which were now very dark and refractive and which were beginning to decrease in size, although most of them were still large.

From this point on the reaction is obviously related to the events that took place in these cells. Monocytes continued to enter the field for seventy-two hours and to be converted through the stages leading to this type of macrophage; tissue clasmatocytes which were not already rounded and free eventually became so and merged indistinguishably with their fellow macrophages. The progression of events, however, is represented by the changes which took place in those cells which had already become macrophages and which were filled with deposits of neutral red and with anisotropic drops. The description of the macrophages from now on, therefore, is indiscriminate of their original source.

While free crystals had completely disappeared from both the field and the macrophages thirty-six hours after the injections, the cells continued to acquire increasing numbers of anisotropic drops, which eventually became arranged several layers deep in the periphery of the cytoplasm (fig. 2, *G* and *H*). These drops were waxy, brilliantly anisotropic and jeweled with crosses under polarized light; they now stained deeply with sudan, slightly with osmic acid and not at all with nile blue sulfate; they reacted in the Schultz test and were largely removed by ether after treatment with digitonin. These anisotropic drops occurred in greatest abundance seventy-two hours after the injection; they began to lessen in number during the next two days (fig. 3, *36°* to *4-5 d*).

The vacuoles stainable with neutral red, in the meantime, became displaced centrally as the anisotropic drops took up a peripheral position, and at the same time they began to decrease in size and to increase in number and uniformity. This change was marked by forty-eight hours, still more marked by seventy-two hours, the period when the anisotropic drops were most abundant (fig. 2, *G* and *H*), and most marked at four and five days, the period when the anisotropic drops were rapidly decreasing (fig. 3, *36°* to *4-5 d*). At this stage the deposits of neutral red approached, but never attained, the fineness of the deposits in typical epithelioid cells. By the sixth and seventh days the drops of lipid had decreased impressively and had lost the cross of polarization but were still anisotropic; the deposits of neutral red were as fine as those of the two preceding days but had also decreased greatly in number. From the seventh day the events were regressive in character (fig. 2, *I* and fig. 3, *5-10 d*). All fat and the fine, dark deposits of neutral red soon disappeared and were replaced by scattered deposits of neutral red which were more neutral in reaction, larger, angular and more like the deposits in normal tissue clasmatocytes (fig. 2, *J*). The cells elongated and decreased in number. By this time their only reaction to the fat stains consisted of stippling with sudan IV similar to the stippling of tissue clasmatocytes.

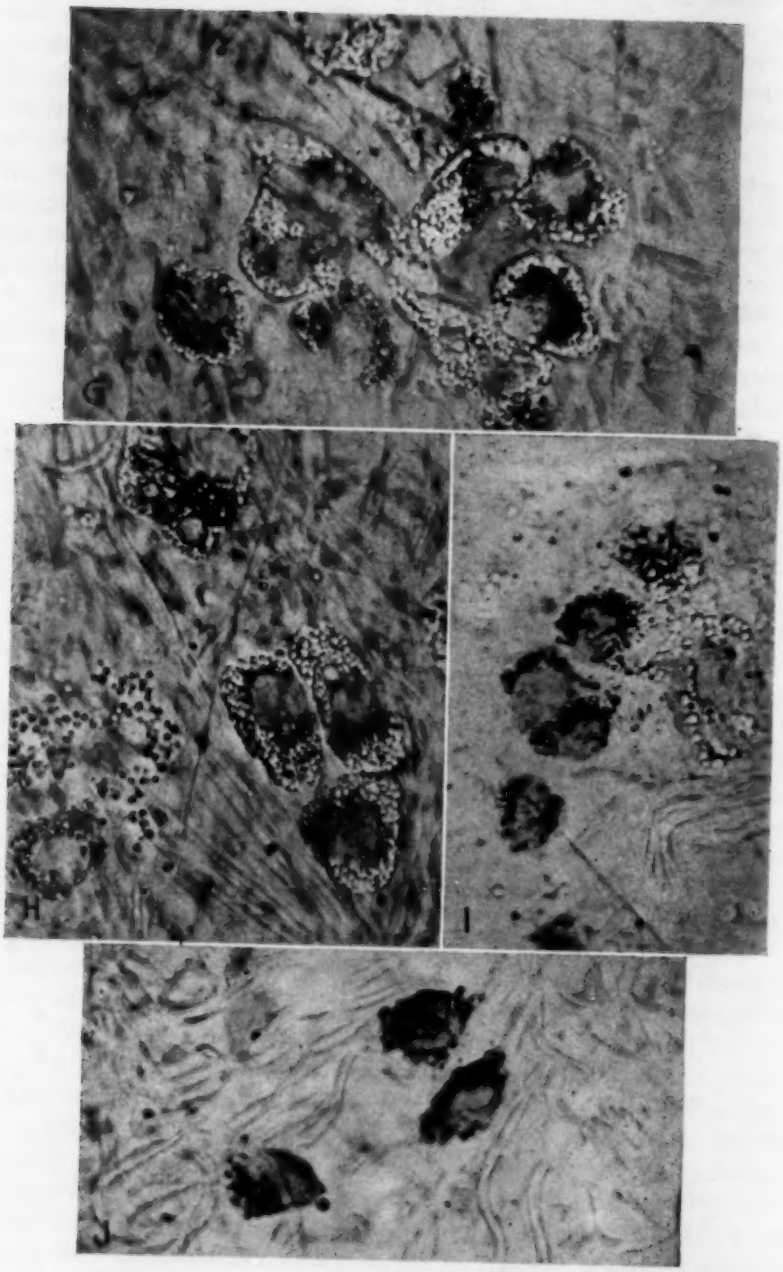


**Figure 2**

*(See legend on opposite page)*

While observations were continued much longer, the foregoing description seems to represent the sequence of events consecutively from start to termination following a single injection of cholesterol in colloidal suspension. Cytologic death occurred wherever cells were massed into closely packed foci, and this was followed by chemical reorganization with deposition of large plaques and needle-like crystals, together with the fats from cellular autolysis. The sequence of events following a single injection of cholesterol in colloidal suspension was subject to constant repetition in such areas but was modified by foreign body and fibrous reactions to the constituents of the foci. The cells in the centers of the foci were typical of the macrophages observed about forty-eight hours after the experimental injections. They were swollen with large anisotropic drops, which contained crosses of polarization and stained faintly with sudan. They were typical of the cells following injections of cholesterol in crystalline form described by earlier workers. They were obviously not end stages but intermediate stages (of the sequence of events just described) in areas where autolysis and crystalline depositions prolonged the processes of absorption and reaction. All the stages leading to the morphologic alterations described at three, four and five days after the experimental injections could be found along the axes leading outward from the foci, and thence outward were to be found the stages toward regression and resumption of the normal.

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EXPLANATION OF FIGURE 2.

The introductory comments on the films shown in figure 1 apply here except that crystals were no longer present.

*G.* Mouse 87. Abdomen. Sufficient cholesterol was injected seven days previously to prolong the reaction for photographic purposes. This reaction is characteristic of the experimental reactions at three to four days. Tissue clasmatocytes and macrophages had become indistinguishable and occurred in masses. They were characterized by accumulations of anisotropic drops of lipid at the periphery of the cytoplasm and by the extreme uniformity, smallness and dark hue of the deposits of neutral red. The latter were abundant and were located central to the drops of lipid. The lipid contained crosses of polarization under polarized light.

*H.* Same as *G.*

*I.* Mouse 88. Abdomen. A colloidal suspension of cholesterol was injected eight days previously. Macrophages were in various stages of regression. The one at the right still contained considerable lipid. The three middle ones had lost practically all lipid, but still contained many deposits of neutral red, which had the characteristics of those illustrated in *G* and *H*. The upper and lower cells were intermediate in character. They still contained some lipid.

*J.* Mouse 88. Back. Cholesterol in colloidal suspension was injected ten days previously. These three macrophages had practically finished the stages of regression. The two upper ones contained almost no fat and much less neutral red than previously. The lower one contained no fat. The fine, dark, uniform deposits of neutral red which were characteristic of the earlier periods had disappeared, and the deposits which were present were larger and more neutral in reaction than in the preceding periods. The cells were similar to normal tissue clasmatocytes.

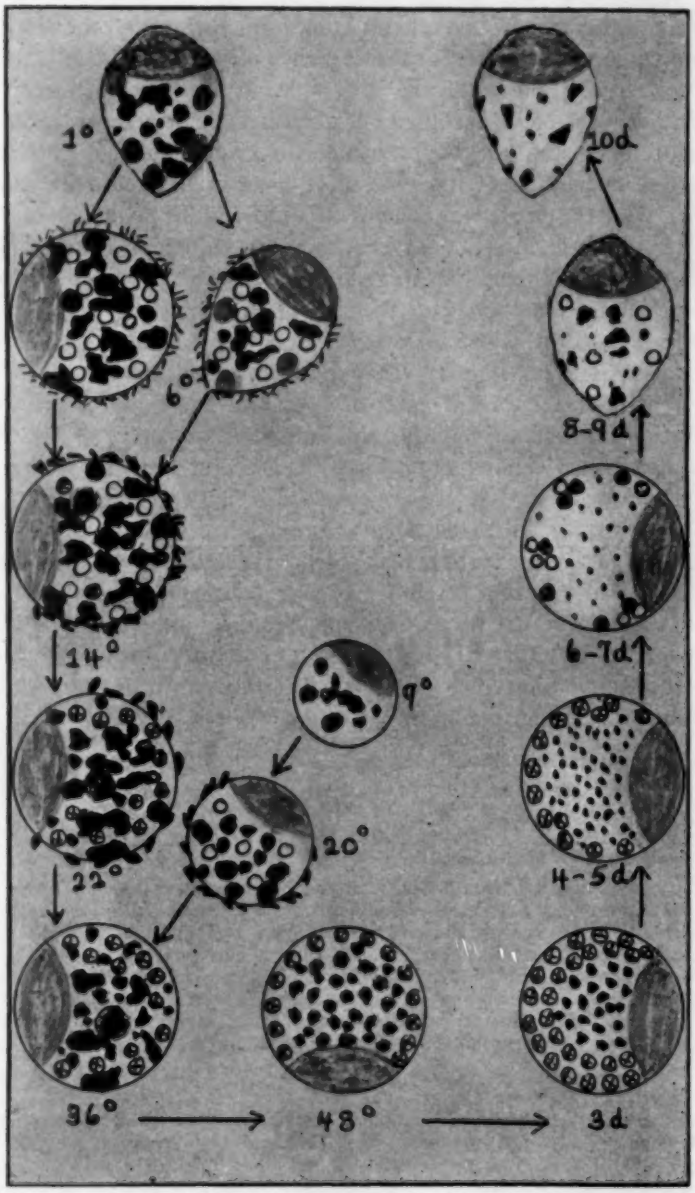


Fig. 3.—Schematic representation of the sequence of events observed in reticuloendothelial cells after cholesterol in colloidal suspension had been injected subcutaneously. The stated numbers of hours (1°, 6° and so on) and days (d) are merely indicative of the intervals after injection at which cells with any specific morphologic character began to occur with relative frequency. Comments on the character of the staining with sudan IV are added for comparison, but the staining with sudan IV is not represented.

*(Legend continued on opposite page)*

## Explanation of Plate.

Solid gray or black areas represent deposits of neutral red of varying depths of color.

Empty circles represent drops of isotropic fat.

Cross-hatched circles represent drops of anisotropic lipid without crosses of polarization.

Circles with crosses represent drops of anisotropic lipid with crosses of polarization.

*1 hour.* The tissue clasmatocyte is slightly contracted. It contains neutral red vacuoles, "fluid vacuoles" and metachromatic vacuoles, but neither fat nor cholesterol. (Sudan IV—diffuse stippling only.)

*6 hours.* Some tissue clasmatocytes remain elongated, some have become rounded. The staining with neutral red is similar to that at one hour. Droplets of isotropic fat have become scattered at random in the cytoplasm, and many fine crystals of cholesterol are adherent to the surface. (Sudan IV—diffuse stippling; also a few vacuoles rimmed with orange and a rare solid orange deposit.)

*9 hours.* Monocytes enter the field. They are small and contain neither fat nor crystals of cholesterol. The deposits of neutral red are small and neutral in reaction.

*14 hours.* The deposits of neutral red in the tissue clasmatocytes have become larger, darker and more refractive than at six hours. Lipid droplets are scattered at random in the cytoplasm as at six hours, but some of them are now anisotropic. The crystals of cholesterol adherent to the surface tend to be arranged in parallel and are thicker than at six hours. (Sudan IV—numerous areas that are either rimmed with orange or solid orange.)

*20 hours.* The monocytes have developed to macrophages. At this stage they contain much larger deposits of neutral red than at nine hours. This, however, is still neutral in reaction. Coarse acicular crystals of cholesterol adhere to the surface, and drops of isotropic fat are scattered at random throughout the cytoplasm.

*22 hours.* All of the lipid in the tissue clasmatocytes is now anisotropic, and crosses of polarization are present. The crystals adherent to the surface are much fewer and much coarser than at fourteen hours. They have the shape of grains of rice. The deposits of neutral red are the same as at fourteen hours. Cellular debris has been phagocytosed. (Sudan IV—numerous large, solid orange deposits.)

*36 hours.* The tissue clasmatocytes and the metamorphosing macrophages have become indistinguishable. Crystals of cholesterol have disappeared from the surfaces of both. Both contain anisotropic drops of lipid which exhibit crosses of polarization and are tending to be arranged at the periphery of the cytoplasm. The deposits of neutral red are dark and refractive in both. They are becoming more central in location and slightly smaller. (Sudan IV—more solid orange deposits than at twenty-two hours.)

*48 hours.* The drops of anisotropic lipid with crosses of polarization are now practically entirely peripheral in location. The deposits of neutral red are much smaller than at thirty-six hours, more uniform and more centrally located. (Sudan IV—great numbers of large solid orange deposits.)

*3 days.* The accumulation of anisotropic drops with crosses of polarization is at the maximum. The drops are packed thickly at the periphery of the cytoplasm. The deposits of neutral red are packed more centrally. They are abundant and smaller than at forty-eight hours. They are still dark and refractive. (Sudan IV—maximum number of large solid orange deposits. These tend to have a peripheral location. The cytoplasm central to them is very basophilic.)

*4-5 days.* The anisotropic drops at the periphery of the cytoplasm have begun to disappear. The deposits of neutral red central to them remain abundant and are even smaller than at three days. (Sudan IV—fewer and more scattered solid orange deposits than at three days.)

*6-7 days.* The drops of lipid have almost disappeared. Many are no longer anisotropic. The deposits of neutral red are now disappearing also. They are the same size as at four to five days. (Sudan IV—scattered solid orange deposits mingled with vacuoles which are merely rimmed with orange.)

*8-9 days.* Very few drops of lipid are now present. They are all isotropic. The fine deposits of neutral red have disappeared. The deposits present are now large, irregular in shape, neutral in reaction or buff in color, and less refractive than formerly. The cells are more like normal tissue clasmatocytes than at any time since the injections. (Sudan IV—merely a few vacuoles rimmed with orange.)

*10 days.* The cells are elongated and do not contain lipids. They contain little neutral red, and that is characteristic of the deposits in normal tissue clasmatocytes. (Sudan IV—diffuse stippling only.)

COMMENT

*Technics.*—The limitations and the specifications of the different technics for staining lipids are discussed in various texts on the subject and have been critically analyzed by Lison.[8] Discussion of the technics employed in these experiments seems indicated, therefore, only so far as they apply to the deductions to be drawn from the data presented.

Since all the lipid studies were made either from living or from air-fixed films, the lipids were in as nearly normal antemortem state as possible. The probability of their having been chemically changed, dissolved by fixatives or otherwise modified, which usually has to be taken into consideration in histologic studies of lipids, seems unlikely in these studies.

The supravital preparations permitted a surprising degree of analysis and demonstrated the possibilities inherent in this method for the study of lipids. The technic served in one respect in which the other technics failed entirely. It permitted detection of the tiny acicular crystals of cholesterol which were free in the field and adherent to surfaces of the tissue clasmatocytes and macrophages up to thirty-six hours after the injections. The crystals did not stain by any technic. They were so small and their refractive index was such that they were not discernible in the mounts of glycerin jelly, nor was the space occupied by them usually demonstrable after mounting in balsam. On the other hand, they were strikingly evident when observed supravitally by their refractivity. They were too delicate to be definable as separate crystals by either the Schultz or the digitonin technics.

The brilliant refractivity of the unstained drops of lipid in the supravital preparations made them, also, demonstrable at a smaller size and at earlier periods than was possible with any of the fixation technics employed. This was particularly striking in the studies of fibroblasts, degenerating polymorphonuclear cells and young macrophages. Single droplets were often brilliantly refractive in the supravitally stained films at early hours after injection, when the companion fixed films had not yet begun to stain by any of the other technics.

Not only were the lipid droplets demonstrable in the living films, but they could be clearly separated into isotropic and anisotropic lipids at either body or room temperature. If isotropic, the droplets obviously consisted of either fatty acids or triglycerides.[8] The fact that blue deposits were absent after staining with nile blue sulfate throughout the entire experiment seems to rule out the possibility of free fatty acids. When the droplets became anisotropic, they obviously contained other lipids. Demonstration of the cross of polarization was facilitated

8. Lison, L.: Bull. d'histol. appliq. à la physiol. **10**:237, 1933.

by the absence of fixation.[8] The phenomenon, when present, delineated the lipid into esters of cholesterol, vs. free cholesterol, or into phospholipids or glycolipids.

The Schultz technic is destructive of cytologic detail and therefore permits detection of the cholesterol radical merely grossly, rather than as specific deposits within cells. It does not permit differentiation between cholesterol and its esters. However, most contributions concerning the histochemical detection of cholesterol and its esters assume that a cross of polarization occurring in the presence of this radical indicates the presence of the esters rather than of free cholesterol. The truth of this assumption seems to have been satisfactorily determined by Adami and Ashoff.[9] In these experiments, therefore, the Schultz technic served to demonstrate cholesterol-containing material within the cells, while the cross of polarization indicated that it was in ester form.

These deductions were corroborated by the studies with digitonin. That technic proved to be serviceable only when cholesterol or its esters were present in sufficient amounts for detection. Formation of large enough particles of the sterol-digitonide for certain detection under polarized light and separation from esters was possible only after the colloidal particles of cholesterol had aggregated or after the cells became loaded with esters. At the early hours after the injections, when the colloidal particles were free or adherent to the tissue clasmatocytes, the complex was rarely discernible. In the later hours, however, the films exposed to digitonin served to demonstrate that cholesterol surrounded the cells and that esters filled them. At still later periods, i. e., at four or more days, the films served to demonstrate that both esters and cholesterol had been secondarily deposited as large masses in the foci of packed cells.

Detection of the intracellular deposits of lipid by sudan IV was more sensitive than that by any of the other technics except study of the living films. The heavily loaded cells in the centers of the packed foci stained only slightly, but elsewhere the cells stained deeply; the former obviously must have consisted largely of esters of cholesterol, while the latter contained generous components of neutral fats. The tinting of the intracellular deposits with osmic acid indicates that oleic or other unsaturated fatty acids were present in moderate amount either in the component of neutral fat or as an ester of cholesterol.

In summary, then, the supravital preparations served to demonstrate the presence and the time of disappearance of acicular crystals of cholesterol, both free and on the cell surfaces. They served to demonstrate the presence of isotropic lipids, i. e., neutral fats or fatty acids, in the cells before the anisotropic lipids, i. e., cholesterol esters, became demonstrable and before reaction to lipid stains became positive.

9. Adami, J. G., and Ashoff, L.: Proc. Roy. Soc., London, s.B **78**:359, 1906.

Finally, they served to demonstrate anisotropism and crosses of polarization with greater surety than was possible in fixed films. Histochemical technics with fixed films were necessary to demonstrate the presence of the cholesterol radical in the lipid vacuoles versus that of other anisotropic lipids. The occurrence of the cross of polarization indicated that this was in ester form.

*Analysis of the Deductions from the Data Concerning Lipids.*—If the foregoing analysis of technics may be accepted, the sequence of events following the presentation of colloidal suspensions of cholesterol to the tissues seems to have been as follows: The scattered chylomicron-like globules of cholesterol in the suspensions were deposited as discrete fine acicular crystals as soon as the menstruum was absorbed (a matter of about three hours); the crystals were electively attracted to the surfaces of any tissue clasmatocytes in the area and to those of the macrophages which entered later. Isotropic fat appeared in the clasmatocytes and the macrophages slightly before anisotropic lipid was demonstrable. The crystals gradually increased in size even as they were gradually disappearing. They grew to the shape of grains of rice before all had disappeared. Disappearance seemed to take place mostly on the cell surfaces, although it cannot be stated that free crystals did not disappear without adhering to cells. As the crystals disappeared, the drops of lipid within the cells increased, became anisotropic and acquired crosses of polarization. According to the preceding discussion of technics, the crystals of cholesterol were converted to esters and entered the cells as such. All crystals had disappeared within thirty-six hours. The anisotropic drops of cholesterol esters acquired a peripheral position within the cells and continued to increase in number up to seventy-two hours. They gradually decreased thereafter. Few refractive droplets of lipid were present within the macrophages by six days after the injections, and, of these, most were no longer anisotropic.

Whether esterification of the colloidal cholesterol occurs only on the surfaces of clasmatocytes and macrophages, or in the intercellular fluid also, is not definite, but seems likely. Shope,[10] Sperry[11] and Schönheimer and Yuasa[12] have demonstrated an enzyme capable of esterifying cholesterol in connective tissues, but the technics which they employed do not permit conclusion as to whether it is intracellular or extracellular in location. The fact that both fibroblasts and polymorphonuclear cells in the area acquired droplets of neutral fat and yet at no time acquired anisotropic droplets, i. e., cholesterol esters, would lead to the opinion that either these cells, in contrast to tissue

10. Shope, R. E.: J. Biol. Chem. **80**:127, 1928.
11. Sperry, W. M.: J. Biol. Chem. **113**:599, 1936.
12. Schönheimer, R., and Yuasa, D.: Ztschr. f. physiol. Chem. **180**:19, 1929.

clasmatocytes and macrophages, are impermeable to cholesterol esters in their environment or that the esters were formed only at the surface of the reticuloendothelial cells by virtue of esterase within these cells. The latter explanation seems the more likely. Correlation of these observations lends probability to the concept that while the presence of neutral fats is necessary if cholesterol is to be converted to its esters, the process of conversion takes place on the surfaces of cells of the reticuloendothelial system and not in the tissue spaces.

*Correlation of the Sequence of Events in the Lipid Vacuoles and the Sequence of Changes in the Cytoplasmic Elements Which Stained with Neutral Red.*—The terms "neutral red vacuoles," "phagocytic vacuoles," "digestive vacuoles," "excretory vacuoles" and "supravital vacuoles" have been used rather indiscriminately and synonymously in most discussions concerned with the supravital technic. The present studies with cholesterol emphasize that the areas in phagocytic cells which stain with neutral red may differ considerably from the areas in which materials are first phagocytosed and deposited within the cytoplasm. In other words, the truly phagocytic vacuoles and the neutral red vacuoles may, or may not, be independent structures. In these studies the vacuoles which contained esters of cholesterol did not stain with neutral red. They can obviously be regarded as phagocytic vacuoles. On the other hand, the elements which stained with neutral red went through metamorphoses as striking as those of the lipid vacuoles and characteristically correlated with them in time. It would seem, therefore, that the areas stained with neutral red may be regarded as the truly digestive and excretory areas. In support of this concept is the fact that these areas were not demonstrable by the technics for lipids, did not vacuolate after exposure to fat solvents and stained simply as a part of the general cytoplasm in the fixed films. It would seem, therefore, that they represent specialized cytoplasmic elements with the chemical reactions of cytoplasm, i. e., of proteins, but capable of specific supravital staining.

The neutral red deposits of the tissue clasmatocytes changed characteristically soon after the injections. The same was true of the deposits in the macrophages as soon as those cells developed. The deposits increased greatly in size and number, became darker red, i. e., more acid, and more refractive than normal, and the cells containing them became swollen and rounded. These changes were more or less synchronous with the appearance of isotropic droplets within the cells and preceded the appearance of anisotropic material. They continued, however, after onset of the latter event. As the free crystals of the cholesterol decreased in number on the surfaces of the cells and the anisotropic drops containing the cross of polarization increased within the cells, the elements stained with neutral red gradually

decreased in size, even while they increased in number in the process. They remained dark in color. They thus became strikingly uniform in both color and size. At the same time they became centrally located in the cells, and arranged as a large rosette in the area of the centrosome, which was framed by the anisotropic drops of cholesterol ester at the periphery of the cytoplasm. These changes in the deposits of neutral red began as early as thirty-six hours after the injections. The fineness and the uniformity of the deposits were striking by seventy-two hours, the period at which the greatest number of anisotropic drops occurred. The deposits remained abundant but continued to decrease in size during the next two days, when the anisotropic drops were decreasing in number. At this time the cells gave every indication of becoming epithelioid in type. Decreases in the size of the neutral red deposits, however, did not progress beyond that attained on the fourth and fifth days after the injections. The deposits then gradually disappeared without attaining the dustlike fineness of those in epithelioid cells and without the development of binucleated cells or other evidences of cellular degeneration. In this respect the cellular reaction was similar to the response which Gray [2] observed in guinea pigs after injections of tributyrin. Disappearance of the deposits of neutral red followed on the disappearance of the droplets of cholesterol ester. By eight days any deposits of neutral red had become scattered, larger in size, lighter in color, angular in shape and much like the deposits in normal tissue clasmatocytes.

Correlation of the changes in the neutral red deposits with the changes in the droplets of cholesterol ester suggests the possibility that the former are directly related to the latter and represent end stages in a series of events beginning at the cell surface and progressing toward the completed stage represented by the special cytoplasmic elements which stained with neutral red in the area of the centrosome. The fact that the peak of events in the deposits which stained with neutral red occurred later, and ended later, than the peak of events in the lipid droplets lends support to this concept. If this concept is correct, one may conceive that the neutral red deposits in these experiments were end products of the digestion of the cholesterol esters, and of such nature that they had lost the ability to react to fat stains or to be dissolved from the cytoplasm by the usual fat solvents. That is to say, they behaved in the manner of the lipoprotein elements of cells, which, in this case, would seem to be represented by one of the combinations of protein and cholesterol. A discussion of these has recently been presented by Bloor.[13]

13. Bloor, W. R.: Biochemistry of the Fatty Acids and Their Compounds, the Lipids, American Chemical Society Monograph Series, New York, Reinhold Publishing Corporation, 1943.

The reticuloendothelial cells are obviously able to attract cholesterol that is in a colloidal state and probably are able to convert it into esters. They are certainly able to absorb it as esters. Further, having absorbed the esters, they are able to handle them so that they ultimately disappear without injury to the cells unless the cells themselves are handicapped by too great a degree of crowding. If the theory presented in this study is tenable, they are converted into combinations of cholesterol and protein within the individual cells, and excreted as such.

*Literature.*—The findings about the foci of necrotic masses of cells at considerable periods after injections of the colloidal suspensions of cholesterol, and the findings after injections of concentrated emulsions with more or less aggregation, as well as the findings of earlier workers who employed crystalline cholesterol suspensions of varying strengths, are in contrast to the primary reactions observed with colloidal cholesterol suspensions as they have been presented. In the former instances the coarse needle-like or plaquelike crystals deposited were surrounded by giant cells as well as by mononuclear cells similar to the macrophages which occurred three or four days after the injections of the colloidal suspensions. These reactions were intermingled with necrosis and proliferation of fibrous tissue. Responses of this type represent a mixture of reactions. One is the foreign body reaction, or the reaction to large insoluble material. The other is a reaction essentially like that described after the injections of cholesterol in colloidal suspension. It is represented by the large cells filled with anisotropic drops which were long recognized as cholesterol esters. That these cells were continually present at late periods rather than in the regressive stages described in the present experiments seems to be due to the fact that cholesterol was being constantly presented to them as the large crystals were slowly dissolved in the fats liberated in the processes of necrosis. The reaction may be considered to have been repeating itself indefinitely until all of the crystals could be dissolved and absorbed by the cells. Necrosis apparently took place in the process, however, and sufficient neutral fat and fatty acids were liberated to stimulate proliferation of fibrous tissue. The early workers in this field examined the reaction only after it had reached this stage (Basten,[14] LeCount,[15] Schönheimer and Yuasa,[12] Reiss[16] and Kimmelstiel and Laas[17]). It is only recently that the differences in the reaction to cholesterol in the colloidal and in the crystalline state are beginning to

14. Basten, G.: Virchows Arch. f. path. Anat. **220**:176, 1915.

15. Le Count, E. R.: J. M. Research **7**:166, 1902.

16. Reiss, H.: Virchows Arch. f. path. Anat. **296**:627, 1936.

17. Kimmelstiel, P., and Laas, E.: Beitr. z. path. Anat. u. z. allg. Path. **93**:417, 1934.

be appreciated. Both Hirsch [18] and Weinhouse [19] have suggested that the reaction to cholesterol in colloidal form is the more dynamic reaction of the body, and of very different import from the reaction to cholesterol in crystalline form.

These studies, then, lead to the conclusion that the cells of the reticuloendothelial system are specifically constituted to handle cholesterol. They react to it in one way when it is presented in solid form, and in another when it is presented in colloidal form. The latter seems to represent the specific, direct reaction by which cholesterol is absorbed from the tissues, modified, combined and excreted back into the body fluids. The reticuloendothelial cells in the subcutaneous tissue that take part in this reaction are from two sources, i. e., the preexistent tissue clasmatocytes and the macrophages that develop from monocytes. Both types of cells react the same and eventually become indistinguishable. Both electively attract the acicular crystals of cholesterol. The cholesterol is converted into esters at the cell surfaces. The esters are absorbed and segregated as liquid crystals in vacuoles at the periphery of the cytoplasm. The elements which stain with neutral red and which are more centrally located in the cytoplasm pass through a series of transitions which suggest conversion of the esters into combinations of cholesterol and protein and their ultimate excretion in that form. After completion of the stages outlined, with disappearance of the fine deposits of neutral red that have been evolved in the process, the cells regress to a morphologic state which is like that of normal tissue clasmatocytes and decrease in number. The time required for this complete cycle is considerable, and undue crowding of the reactive cells may result in sufficient interference with nutrition and absorption to cause them to succumb. The reactions which follow in that event are no longer characteristic of the response to cholesterol alone.

## CONCLUSIONS AND A HYPOTHESIS

Cholesterol in colloidal suspension is deposited subcutaneously as discrete acicular crystals which are electively attracted to the surfaces of cells of the reticuloendothelial system. These are represented by both preexisting tissue clasmatocytes and macrophages which develop from hematogenous cells.

The acicular crystals are converted to cholesterol esters and enter the cells. It seems, but is not definitely proved, that this process occurs only at the cell surfaces, by virtue of esterase within the cells, and not in the interspaces.

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18. Hirsch, E. F.: Arch. Path. **25**:35, 1938. Hirsch, E. F., and Weinhouse, S.: ibid. **30**:1097, 1940.

19. Weinhouse, S.: Arch. Path. **35**:438, 1943.

The esters become segregated in the peripheral part of the cytoplasm as liquid crystals. They gradually disappear, their disappearance being concomitant with characteristic changes in the elements which stain with neutral red.

The elements stained with neutral red first enlarge and become refractive and acid in reaction. They assume central location as the esters accumulate in the peripheral part of the cytoplasm, decrease in size, increase in number and remain acid in reaction. These changes are followed by rapid dissolution and regression after the esters have disappeared.

The hypothesis is presented that the elements which stain with neutral red in these experiments represent combinations of cholesterol and cell proteins, and are excreted as such.

High concentrations of cholesterol or aggregations of cells crowded to the point of necrosis in areas reacting to a colloidal suspension of cholesterol result in crystalline depositions. In that case foreign body and fibrotic reactions merge with a continued response to the cholesterol in colloidal form.

# SYSTEMIC MULTICENTRIC LIPOBLASTOSIS

C. G. TEDESCHI, M.D.
Director of Laboratories, Framingham Union Hospital
FRAMINGHAM, MASS.

NO GROUP of tumors has more successfully resisted attempts at orderly arrangement than those of the lipid system. The pertinent problems are the same as those which obscure the embryogenesis of normal fat tissue; further difficulties arise from the frequent overlapping of morphologic aspects in the great variety of conditions in which overgrowth of fat tissue occurs. For these reasons the attempt to establish the nature of an unusual case of multiple recurrent fat tissue growths leads to the consideration of various related pathologic conditions.

## SURVEY OF LITERATURE

The distinction generally drawn between obesity, multiple lipoma and lipomatosis in its several varieties—"symmetric diffuse," "dolorosa," "discrete" and "cervical" (Mosny and Beaufumé [1] and Madelung [2])—does not always appear to be justified on morphologic grounds. Wells [3] cited cases of multiple lipoma accompanied with tenderness such as is found in adiposis dolorosa (Dercum's disease). He also recalled cases classified as instances of sex infantilism with obesity (adiposogenital dystrophy, or Fröhlich's syndrome) in which later the condition merged into generalized obesity or diffuse lipomatosis. The case observed by Vallery-Radot and associates [4] of a young woman who displayed in succession a circumscribed lipoma of the thigh, then obesity and subsequently huge fatty nodules in the subcutaneous tissue further exemplifies the ill defined limits among "lipopathies."

Symmers and Fraser [5] described a condition occurring in marantic infants which was attended by hyperplasia of the so-called primitive fat organs so pronounced as to resemble a neoplastic growth. They also recognized a group of chronic productive inflammatory conditions accompanied with hyperplasia of embryonal fat cells which infiltrate the

1. Mosny and Beaufumé: Bull. et mém. Soc. méd. d. hôp. de Paris **19**:106, 1902.

2. Madelung, O. W.: Arch. f. klin. Chir. **37**:106, 1888.

3. Wells, H. G.: J. A. M. A. **114**:2177, 1940.

4. Vallery-Radot, P.: Blamoutier, P., and Krief, J.: Bull. et mém. Soc. méd. d. hôp. de Paris **48**:1083-1086, 1924; quoted by Coormaghtigh.[21]

5. Symmers, D., and Fraser, A.: Arch. Int. Med. **19**:699, 1917.

surrounding tissue in a neoplastic fashion. Their observations remind one of the so-called intestinal lipodystrophy (lipophagia granulomatosis), in which lipids are deposited in the mucosa of the small intestine and lipid-laden mononuclear cells are present in the mesenteric lymph nodes, simulating cancerous lipoblastoma.[6]

In addition, there are reports in the literature of diffuse lipomatosis which became locally progressive and exhibited wild potentialities of growth (Robertson [7]). The opposite course was recorded by Mirolli [8] in his report of a case of long-standing diffuse lipomatosis, in which he observed gradual regression of the newly formed masses of fat tissue.

In spite of its definitely neoplastic characteristics, even a mature lipoma presents an intimate structure which does not differ basically from that of a nonencapsulated fatty overgrowth. Wells [9] has emphasized that the mature lipoma and the steatopygous deposits of the Hottentot women arise in the same way, from embryonic mesenchymal cells of the preadipose tissue; this same histogenesis has been advocated for the so-called heterologous lipoma (Chiari [9]) and also applied to the fatty nodules occasionally encountered in the subcutaneous adipose tissue, often mistaken for lipoma, and defined by Mallory [10] as "nonencapsulated moruloid tumors of the adult adipose tissue."

Immature cells appearing in a fatty growth may lead to the false conception of a cancerous process (Jaffé [11]). On the other hand, an entirely mature lipoma, arousing no untoward suspicion from the histologic standpoint, may be the site of repeated recurrences. Ewing [12] reported a case in which a lipoma of the spermatic cord recurred many times in the course of fifteen years in spite of the consistently mature appearance of the fat cells on repeated histologic examinations. A retroperitoneal lipoma described by Horn [13] displayed a similar behavior, and identical observations by Winkler,[14] Hosemann,[15] Lang,[16] Labey,[17]

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6. Whipple, G. H.: Bull. Johns Hopkins Hosp. **18**:382, 1907. Blumgart, H. L.: Arch. Int. Med. **32**:113, 1923. Jarco, S.: Bull. Johns Hopkins Hosp. **59**:275, 1936. Reinhart, H. L., and Wilson, S. J.: Am. J. Path. **15**:483, 1939. Sailer, S., and McGann, R. J.: Am. J. Digest. Dis. **9**:55, 1942. Hill, J. M.: Am. J. Path. **13**:267, 1937. Pearse, H. E.: Surgery **11**:906, 1942. Fitzgerald, P. J., and Kinney, T. D.: Am. J. Path. **21**:1069, 1945. Amsterdam, H. J., and Grayzel, D. M.: Am. J. M. Sc. **210**:605, 1945.

7. Robertson, H. E.: J. M. Research **35**:131, 1916.

8. Mirolli, A.: Riforma med. **44**:1624, 1928.

9. Chiari, H.: Tr. Chicago Path. Soc. **8**:65, 1910.

10. Mallory, F. B.: The Principles of Pathologic Histology, Philadelphia, W. B. Saunders Company, 1914.

11. Jaffé, R. H.: Arch. Path. **1**: 381, 1926.

12. Ewing, J.: Neoplastic Diseases, ed. 2, Philadelphia, W. B. Saunders Company, 1922.

13. Horn, cited by von Wahlendorf.[84]

*(Footnotes continued on next page)*

Williams [18] and many others seem to justify the conclusion of Seids and McGinnis [19] that such growths should be considered as cancerous regardless of their morphologic aspect. In 4 of 5 cases of Seids and McGinnis in which there were recurrences, mature fat cells prevailed in the growth; the concomitant presence of spindle-shaped or rounded, rather anaplastic cells might, however, be considered as an evidence of a potential cancerous tendency.

In reporting 2 cases of recurrent lipomatous growth of the groin, Jaffé [11] discussed the concept of cancer in lipoblastoma. Sarcomatous change within the stroma of a lipoma does not fulfil in his opinion the qualifications of "liposarcoma." From the group of tumors diagnosed as liposarcoma he also excluded the mixed tumors in which fat tissue is associated with other types of tissue exhibiting evidence of cancer but in whose growth the fat tissue per se seems to play a passive role. He also doubted the sarcomatous nature of the capsulated lipoblastoma which, although composed of embryonic fat cells (lipoblasts), shows little evidence of cellular anaplasia and in which, as a rule, mitotic figures are absent. The term "liposarcoma" is reserved, accordingly, for the growths showing anarchy of fat storage cells, frequent recurrences, invasive power and high metastasizing tendency.

Tumors classified as liposarcoma, in the strict meaning of the term, are in turn subdivided by Ewing [20] into two varieties: an adult fat cell type, composed of granular cells simulating closely those found in chronic inflammation of fat tissue, and an embryonal fat cell type, characterized by incomplete differentiation of perivascular mesenchymal cells which, instead of developing into mature fat cells, stop in early phases of their maturation and exhibit either myxomatous or foamlike properties. Transformation from one cytologic type to another is not rare. Lang [16] has reported a case of primary retroperitoneal lipomyxoblastoma which on recurrence showed the patterns of a mature lipoma to revert to the original lipomyxomatous structure on second recurrence.

From the standpoint of localization, conditions diagnosed as lipoblastoma and lipoblastosis, respectively, are divided by Goormaghtigh and colleagues [21] into two groups, the ones arising in the subcutaneous tissue and the ones arising in the internal cavities and deep organs. Lipoblastosis bears to lipoblastoma, according to them, the same relation

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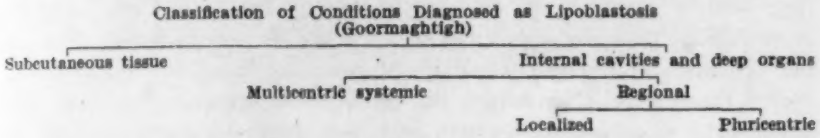
14. Winkler, A.: Ergebn. d. allg. Path. u. path. Anat. **23**: 22, 1930.
15. Hosemann, G.: Arch. f. klin. Chir. **155**: 336, 1929.
16 Lang, W.: Arch. f. klin. Chir. **155**: 349, 1929.
17. Labey: Presse méd. **42**: 1775, 1934.
18. Williams, C.: J.A.M.A. **105**: 195, 1935.
19. Seids, J. V., and McGinnis, R. S.: Surg., Gynec. & Obst. **44**: 232, 1927.
20. Ewing, J.: Arch. Surg. **31**: 507, 1935.
21. Goormaghtigh, N.; Vanderlinden, P., and de Puysseleyr, R.: Cancer, Bruxelles **13**:3, 1936-1937.

that diffuse fibromatosis holds to fibroma. Among the conditions diagnosed as lipoblastosis of the internal cavities, the authors distinguish fat tissue proliferations in a disorderly multicentric distribution affecting different parts of the body (multicentric systemic lipoblastosis) and fat tissue proliferations, either single or multiple, strictly limited to one region of the body (regional, localized or pluricentric lipoblastosis).




The retroperitoneal space is listed as the most frequent site of the localized regional growths, the perirenal region being the oftenest affected. The intermuscular spaces come second in order of frequency. The tendency of the new growths with intermuscular localization to follow the course of the peripheral nerve trunks has made some consider the possibility that there is an anlage of preadipose tissue in the nerve sheaths.[22] The observations of Alsberg,[23] of multiple lipoma and neurofibroma in the same person, and that of Leven,[24] in which the two processes were seen concomitantly in two generations of one family, seem to support this contention. Adair, Pack and Farrior [25] went so far as to consider multiple lipoma as neurolipoma.

Bony localization is third most frequent, according to Goormaghtigh,[21] who recognized benign lipoblastoma, composed of mature fat cells, as in the cases of Cornil and Ranvier,[26] and cancerous lipoblastoma, characterized by cellular atypism, radiosensitivity and tendency to metastasize early throughout the body, as in the cases of Stewart,[27] Barnard [28] and Fender.[29]

The occurrence of localized fat tissue growths in the thoracic cavity is more rarely seen; it is illustrated in the case of Narr and Wells.[30]

The abdominal cavity is still the leading site for the pluricentric regional growths. In the cases of Hirsch and Wells [31] the sites of fat

22. Delachanal, J.: Des tumeurs malignes du tissue cellulo-adipeux, Thesis, Lyon, no. 22, 1910. Bland-Sutton, J.: Tumours, Chicago, W. T. Keener & Co., 1907. Mesa, C.: Prensa méd. argent. **26:** 1779, 1939.

23. Alsberg, A.: Ueber Neurolipome, Inaug. dissert., Berlin, G. Schade, 1892.

24. Leven: Dermat. Wchnschr. **87:** 1563, 1928.

25. Adair, F. E.; Pack, G. T., and Farrior, J. H.: Am. J. Cancer **16:** 1104, 1930.

26. Cornil, V., and Ranvier, L.: Manuel d'histologie pathologique Paris, F. Alcan, 1901, p. 393.

27. Stewart, F. W.: Am. J. Path. **7:** 87, 1931.

28. Barnard, L.: Arch. Surg. **29:** 560, 1934.

29. Fender, F. A.: Am. J. Path. **9:** 909, 1933.

30. Narr, F. C., and Wells, A. H.: Am. J. Cancer **18:** 912, 1933.

31. Hirsch, E. F., and Wells, H. G.: Am. J. M. Sc. **159:** 356, 1920.

tissue proliferation were the retroperitoneal space and the mesentery and other peritoneal folds; in Martland's case [32] the pelvis, the retroperitoneal space, the perirenal region, the mesentery of the small intestine and the gastrocolic omentum were involved by the proliferative process. A diffuse anlage extending from the space of Retzius to the kidneys along the ureters in the retroperitoneal space might perhaps explain, as suggested by König,[33] the tendency of the tumors at this site to grow more rapidly than elsewhere and their cancerous potentialities. Among the 176 cases of retroperitoneal lipoblastoma analyzed by von Wahlendorf [34] there were 21 in which the growth had become cancerous, and among 115 in which the growth had been removed there were 15 in which it recurred in a short time.

Multicentric regional fatty growths are less frequently found in the thoracic cavity. In the case of Rokitansky [35] the growths were located along the intercostal spaces. Two independent lipomatous masses composed of grapelike nodules of yellow fat tissue were present in the case of Klemperer and Rabin,[36] which they interpreted on the basis of a differentiation of embryonal mesenchymal cells into fat cells.

The multiple independent but strictly regional growths in the instances cited represent a connecting link between isolated lipoma and multicentric systemic lipoblastosis, with which the present study is concerned more directly. Only 6 cases of this type [37] have been found on review of the literature; their main clinical and structural peculiarities, together with those noted on personal observation, are summarized in the table. After the details of the present case have been given, its characteristics will be compared with those of the similar cases in the literature, in an attempt at unitarian interpretation.

## REPORT OF A CASE

The study of this case was made possible by Dr. Paul Klemperer, of Mount Sinai Hospital, New York, who also provided the slides for the study of the manner in which fat tissue develops in embryonal and adult life.

F. C., a 34 year old white woman, was first seen in Mount Sinai Hospital, New York, in the summer of 1916. She complained of swelling in the right

32. Martland, H.: Arch. Path. **5**: 932, 1928.

33. König, cited by Goormaghtigh, and others.[21]

34. von Wahlendorf, A. L.: Arch. f. klin. Chir. **115**: 751, 1921.

35. Rokitansky, cited by Goormaghtigh and others.[21]

36. Klemperer, P., and Rabin, C. B.: Arch. Path. **11**: 385, 1931.

37. (*a*) Nienhuis, J. H.: Ztschr. f. Krebsforsch. **22**: 434, 1925. (*b*) Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histolologie, Berlin, Julius Springer, 1925, vol. 6, p. 692. (*c*) Siegmund, H.: Virchows Arch. f. path. Anat **293**:458, 1934. (*d*) Broca: Bull. et mém. Soc. anat. de Paris **25**: 137, 1850. (*e*) Askanazy: Virchows Arch. f. path. Anat. **158**:407, 1898. (*f*) Goormaghtigh and others.[21]

popliteal space, of nine months' duration. Enucleation of the popliteal mass revealed a benign fat tissue tumor. Six years later, in 1922, the tumor recurred. In the meantime a large mass had appeared in the neck. Both the recurring popliteal mass and the new growth in the neck were removed. The pathologic diagnosis was still lipoma; prolific cellularity of the interstitial tissue suggested, however, the possibility that a cancerous change might have occurred. One year later, in 1923, a large lipomatous mass appeared on the right abdominal wall. It was removed in February 1925, together with another recurrent tumor of the right popliteal space. Symptoms of paraplegia appeared in the summer of 1927. The existence of a tumor of the spinal cord was suspected, and operation revealed an extradural fat tumor at the level of the eighth and ninth dorsal vertebrae. At this time evidence was found that the tumor of the neck had recurred. The symptoms of paraplegia, which had disappeared after the removal of the dural growth, reappeared in the fall of the same year. The old laminectomy wound was reopened, and a recurrent extradural fat tumor was enucleated. Numerous subcutaneous tumors developed in the meantime. In the following months the patient showed a gradual decline, and bilateral spastic paralysis with muscular atrophy and loss of sensation appeared in the lower extremities. A third laminectomy revealed recurrence of the old fatty tumor in the right side of the spinal dura. Death occurred twenty days later.

*Postmortem Findings* (summary of autopsy record).—Externally, the outstanding findings were a series of nodular swellings at both sides of the neck, a large mass bulging on the abdominal wall and a similar one in the left thigh, just beneath the Poupart ligament. The mass in the thigh was freely movable and oval in shape, measuring 13.5 by 12 cm. The right popliteal space was almost completely filled by a large, firm, resilient nodular mass, which measured 13.5 by 9 cm. In the back there was a laminectomy incision 14 cm. long at the level of the thoracic portion of the spine, and deep in the wound a sausage-shaped mass was seen to surround the vertebral column from the fifth to the ninth dorsal vertebra and to penetrate the intervertebral foramens.

All these masses showed a similar appearance. They were soft in consistency and had homogeneous and smooth surfaces both externally and on the cut sections. The color was uniformly pale gray, in contrast to the orange-yellow of the surrounding healthy fat tissue. Fine bands of glistening, grayish pink tissue, crossing one another in network fashion, were noticeable here and there in most of the growths.

Other tumor masses, of identical appearance, were found in the internal cavities of the body. One, 3 cm. in the largest dimension, was located beneath the seventh rib; another, about the same size, was embedded in the fat tissue at the bifurcation of the trachea. Three fatty nodules, each the size of a walnut, were present along the course of the left adrenal vessels, and another, apricot sized, was located in the mesentery of the ileum. The mesoappendix and the transverse mesocolon were infiltrated by similar nodular fat tissue growths. A large mass, 8 cm. across, almost completely obliterated the cul-de-sac of Douglas.

Extensive sacral decubitus, with deep ulceration of the skin and severe inflammatory reaction, four stones in the cystic duct and a severe necrotizing pyelocystitis were additional postmortem findings.

*Microscopic Observations.*—Comparative examination of the slides of the popliteal mass that developed first, the slides of the numerous tumor masses removed at subsequent operations and the slides of the masses found at autopsy twelve years later revealed no substantial structural differences. Review of the

| Case and Author | Clinical Course and Symptoms | Localization of the Fatty Growths | Microscopic Features | Diagnosis | Other Significant Findings |
|---|---|---|---|---|---|
| 1. Man, aged 31..... (Broca, quoted by Ewing) | After removal of a lipoma from thigh, hundreds of fat tissue tumors developed; growths remained stationary about 40 yr.; terminal symptoms of dysphagia | Subcutaneous tissue; mesentery, periesophageal region | | | |
| 2. Woman, aged 38.. (Askanazy, 1899) | Patient died a few days after operation on the neck for suspected tumor of thyroid gland | Subcutaneous tissue; breasts, mesentary, axillas and liver | Atrophic fat tissue intersected by bands of fibrous connective tissue; numerous foci of lymphocyte-like cells irregularly scattered | Multiple lipoma | Hyperostosis of skull |
| 3. Man, aged 55..... (Nienhuis, 1925) | Two yr. duration; spastic paraplegia and diabetes | Mesentery, retroperitoneal space, pancreas, mesosigmocolon, pelvis, mediastinum, thoracic vertebrae, sternum, femur, spinal and cerebral dura | Transitional forms from undifferentiated mesenchymal cells to ripe fat cells, the latter prevailing | Lipoblastic sarcoma with metastasis | |
| 4. Man, aged 49..... (Lubarsch, 1925) | ..... | Capsule of left kidney, cortex of left and of right kidney, urinary bladder, serosa of small intestine, esophagus, left lung, myocardium, pelvic and retroperitoneal regions, vertebrae and femur | Mature fat tissue with myxomatous areas; cellular zones within bone marrow fatty growths | Metastasizing lipoma,* the primary source believed to be capsule of kidney | Osteosclerosis of skull and hyperostosis in forehead; calcified cysticercus in right postcentral convolution; internal and external hydrocephalus |
| 5. Woman, aged 65.. (Siegmund, 1934) | Three yr. duration; huge subcutaneous lipomatous masses developed after removal of lipoma from left thigh | Subcutaneous tissue; mediastinum, myocardium, mesentery, serosa of large and small intestines, renal capsule, urinary bladder, pelvis, vertebrae, femur and around abdominal aorta | Transitional forms from embryonal mesenchymal cells to ripe fat cells; fibrous and myxomatous areas | Lipoblastic sarcoma | |

| | | | | | |
|---|---|---|---|---|---|
| 6. Man, aged 59..... (Goormaghtigh, Vanderlinden and de Puysseley, 1956) | Numerous subcutaneous lipomatous growths developed throughout an unspecified period of years | Subcutaneous tissue; upper and lower extremities, right groin, apex of heart (750 Gm.), capsule of right kidney (850 Gm.), medulla of left kidney, great and small epiploon, epiploic appendixes, retroperitoneal space, large intestine, mesentery, diaphragm; 214 independent fatty growths altogether | Transitional forms from undifferentiated mesenchymal cells to ripe fat cells, with a scattering of small lymphocytic-like cells around numerous blood capillaries; fibrous and myxomatous areas | Systemic multicentric lipoblastosis | Acute disseminated tuberculosis |
| 7. Woman, aged 34.. (Tedeschi) | Twelve yr. duration; process started with popliteal fatty tumor diagnosed benign; 6 yr. later recurrence and new fatty growth at neck, both removed and again considered benign; 1 yr. later new growth in abdominal wall and second recurrence of popliteal mass; 2 yr. later development of extradural spinal fat tissue growth causing paraplegia, also first recurrence of growth in neck. After 3 mo. first recurrence of extradural spinal mass and development of numerous subcutaneous tissue tumors; death following removal of second extradural recurrence | Subcutaneous tissue; neck, abdominal wall, thigh, popliteal space, extradural spinal space, thoracic cavity, adrenal region, mesentery, mesoappendix, transverse mesocolon, pelvis | Transitional forms from embryonal mesenchymal cells to ripe fat cells with marked vascularity and foci of extramedullary hemopoiesis | Systemic multicentric lipoblastosis | |

* This case was considered by both Siegmund and Goormaghtigh as diffuse lipoblastosis.

sections from the primary popliteal mass showed it to be composed of mature fat cells, which were supported here and there by a thin interlacing of well vascularized fibrous connective tissue bundles, from which an arborizing capillary network branched into the bulk of the tumor. In between the fat cells there was a scattering of connective tissue cells, mostly fibroblasts and lymphocytes, irregularly mixed with younger mesenchymal cells which showed cytologic characteristics of undifferentiated cells. The latter were irregularly shaped, with occasional cytoplasmic processes, large nuclei, well defined nuclear membranes, scanty amounts of chromatin and one to three nucleoli. Within the cytoplasm of some of these cells were minute fat droplets, orange stained in the sudan III sections, and where the fat droplets were larger and more numerous, the cells assumed, through swelling of the cellular body and withdrawing of the cytoplasmic processes, a polygonal or rounded shape, while the nucleus appeared displaced toward the periphery.

As stated, the sections of the tumor masses removed at subsequent surgical interventions displayed no difference when compared with the primary popliteal growth, and fundamentally identical patterns were shown by the sections from the postmortem material. In the latter most of the growths still consisted of apparently normal fat tissue, but the fat cells varied greatly in size, the supporting stroma was irregular in distribution and there was a distinct increase in cellularity. Among the non-fat-bearing cells, young mesenchymal cells prevailed over less numerous fibroblasts and lymphocytes. These young mesenchymal cells either were sparsely scattered in between the mature fat cells or had coalesced into small nodular formations which, owing to the presence of immature hematic cells (myelocytes and nucleated red blood cells), assumed the appearance of foci of extramedullary hemopoiesis. Fat deposits were seen within the young mesenchymal cells oftener in the sections from postmortem material than in the sections from the surgical specimens. The evidence of cytoplasmic fat storage ranged from minute granule-like droplets to large lipid vacuoles, which on fusion resulted in an adult fat cell with a single large vacuole or several smaller ones bounded by a thin cytoplasmic ring. The nucleus, which had been centrally placed in the early stages of cellular differentiation, appeared sickle shaped, flattened and compressed at the periphery in the matured cell. Even in the most cellular zones there was no evidence of cellular anarchy and no mitotic figures could be seen.

The blood capillaries were abundant and frequently appeared surrounded by thin mantles of young mesenchymal cells. In these vascular areas transitional forms were distinctly observed, ranging from undifferentiated mesenchymal cells to maturing fat cells.

Regressive changes were present here and there and consisted mainly in a pseudomucinous degeneration, as shown by large blocks of eosinophilic structureless material spreading apart and at times overshadowing the proliferated fat tissue cells.

### COMMENT ON CASE AND COMPARISON WITH PREVIOUS CASES

Multiple, recurrent nonencapsulated fat tissue growth, involving in a scattered fashion both the subcutaneous tissue and the internal cavities of the body, was the unusual characteristic in the case here reported, for which, accordingly, the appropriate label seems to be "systemic multicentric lipoblastosis."

The process started in the popliteal space as an apparently benign lipoma, which was removed; the multicentric proliferation of fat tissue began six years later, and, in spite of numerous attempts to control the growths by repeated surgical intervention, a series of recurrences brought the patient to death in the course of twelve years. Among the various localizations the one in the extradural spinal space deserves special consideration, because it must be regarded as the direct cause of death and because of its extreme rarity. Stookey,[38] in reviewing the literature up to 1928, was able to find only 1 case of extradural lipoma, and Elsberg,[39] among 179 extradural spinal tumors, listed a single lipomatous growth, the one described here. Of the six reports of cases of systemic multicentric lipoblastosis summarized in the table, dural localization is mentioned in only one, that by Nienhuis.[37a]

In trying to explain the multiple fat tissue growths, several questions arise: (1) whether the lipomatous masses are the exponent of a widespread process metastasizing from a primary cancerous lipoblastoma or whether they are autonomous and independently originated, and (2) if they should be considered independent, whether they are neoplastic in nature, in the strict meaning of the term, or are the expression of a systemic developmental or dyscrasic disturbance of the lipid system resulting in multiple autonomous growths.

Nienhuis [37a] and Lubarsch [37b] advanced the opinion that the multiple fatty growths in their cases were metastatic. Siegmund,[37c] on the contrary, although labeling his case as one of "lipoblastic sarcoma," favored the conception of a "systemic disease of the fat tissue, the tumoral nature of which is unclear." The opinion of Siegmund was accepted by Goormaghtigh,[21] who interpreted his own case similarly.

Both clinical course and morphologic aspects lead to a similar explanation in the case presented here. The process lasted twelve years after the appearance of the first fatty growth. A rather protracted clinical course is shown also in the other 5 cases of the literature in which the duration of the disease process is known, forty years in the case of Broca,[37d] at least two or three years in the cases of Nienhuis [37a] and of Siegmund [37c] and a long unspecified period in the cases of Goormaghtigh [21] and Askanazy,[37e] the cause of death being attributed by both to an intercurrent infectious process.

In the present case, as in the cases of the others, the gross appearance of the fatty growths was also against the conception of them as cancer; there was, in fact, absence of nodular distribution, generally considered a characteristic of a neoplastic metastasis, and lack of evidence of vascular invasion by the proliferated fat tissue.

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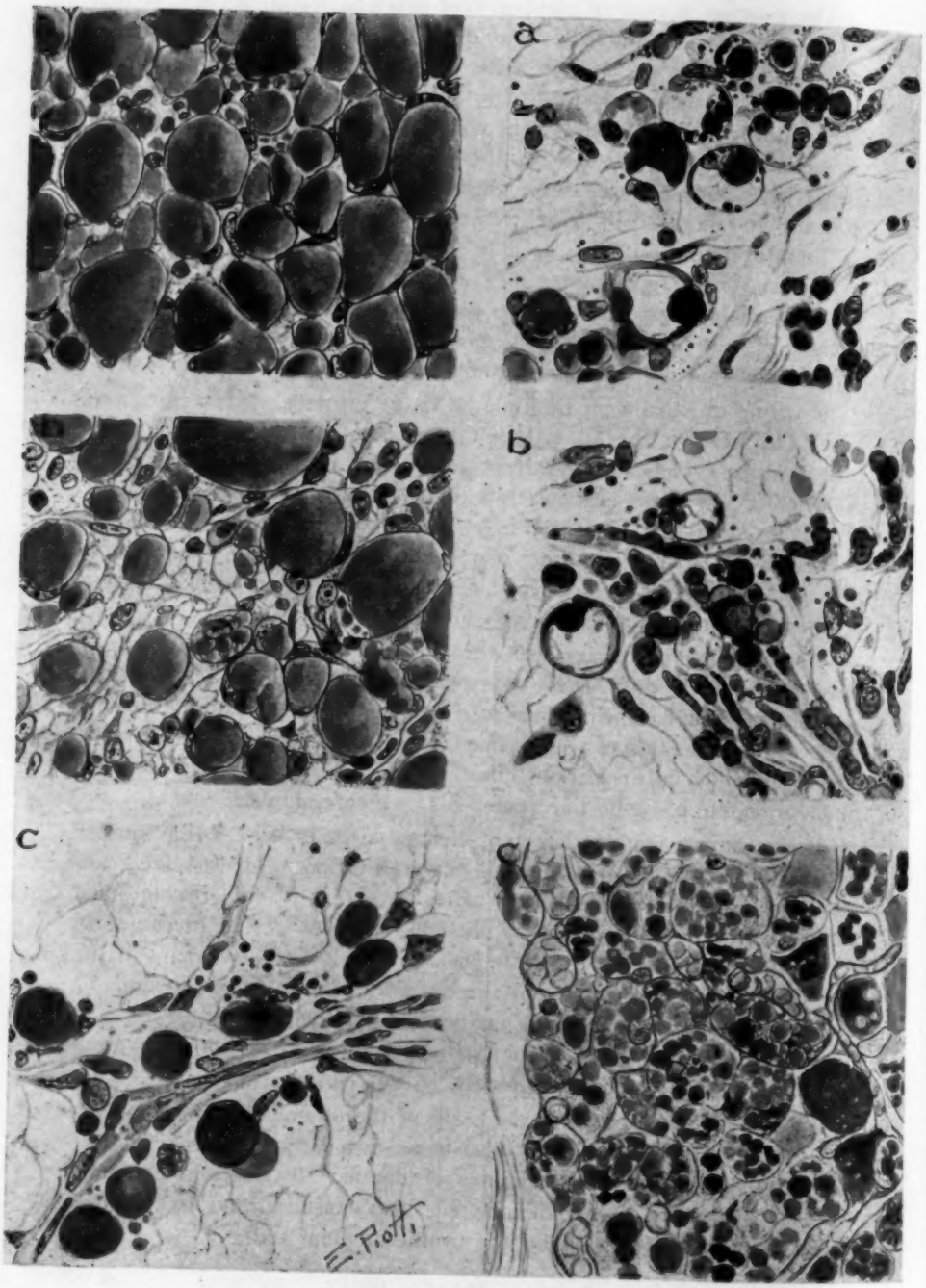
38. Stookey, B.: Arch. Neurol. & Psychiat. **18**: 16, 1926.

39. Elsberg, C. A.: Surg., Gynec. & Obst. **46**: 1, 1928.




*(See legend on opposite page)*

The histologic observations further point against their cancerous nature. In the present case no evidence of cellular anarchy and no mitotic figures could be recognized in the numerous sections of the various fatty growths, which appeared to be mainly composed of adult fat cells, of embryonal fat cells and of less numerous undifferentiated mesenchymal cells, with all intermediate stages. Microscopic patterns of a similar nature are found in the cases of the other authors. In that of Askanazy [37c] and that of Nienhuis [37a] the fatty growths were likewise composed of mature fat cells and of undifferentiated mesenchymal cells, either sparse or in groups, with transitional forms between the two types. In Lubarsch's case [37b] the structure was definitely that of mature fat cells, except for the nodules found within the bone marrow, which appeared unusually rich in young mesenchymal cells. In Siegmund's [37c] and in Goormaghtigh's [21] cases the structure fundamentally was one of irregularly disseminated embryonal mesenchymal cells, which were most numerous around branching blood vessels, with transitional forms from these cells to ripe fat cells.

Additional interest in the present case is offered by the foci of extramedullary hemopoiesis present in most of the growths. This finding raises the question of the role of the embryonal mesenchymal cell of the fat tissue as a potential blood-forming cell (Wasserman,[40] Hübschmann [41] and Gruber.[42] The relationship between blood-forming bone marrow and adipose tissue is well known. That hematic cells are formed within fat tissue in various regions of the body seems to be the rule in

40. Wasserman, J. A.: Ztschr. f. Zellforsch. u. mikr. Anat. **3**: 235, 1926.
41. Hübschmann: Verhandl. d. deutsch. path. Gesellsch. **19**: 236, 1923.
42. Gruber, C. B.: Ztschr. f. Kinderh. **30**: 336, 1921.

EXPLANATION OF FIGURE.

Left (*a*) Mature fat cells supported by a thin interlacing of connective tissue fibers, embedded in which fibroblasts, lymphocytes and younger mesenchymal cells with cytologic characteristics of undifferentiated mesenchymal cells are seen. From the primary popliteal mass removed at operation. (*b*) Mature fat cells showing great variations in size, irregularly intermixed with non-fat-bearing cells, among which young mesenchymal cells prevail over less numerous fibroblasts and lymphocytes. From a fat tissue new growth found in the mesentery at autopsy. (*c*) Mature fat cells being formed from periadventitial mesenchymal cells through increased storage of fat droplets, cellular swelling, withdrawing of cytoplasmic processes and, finally, peripheral displacement and flattening of the nuclei. From a fat tissue new growth, observed in the thoracic cavity at autopsy.

Right (*a*) Early stages of the process by which mesenchymal cells differentiate into fat cells. From a vascular area of the subcutaneous tissue of a human embryo 18 inches (45 cm.) long. (*b*) Progressive stages of fat cell maturation, from the undifferentiated mesenchymal cell containing a few cytoplasmic fat droplets to the ripe fat cell with nucleus displaced to the cell periphery. From the region of the thymus in a 2 day old baby. (*c*) Fat cells in acinous-like arrangement, embedded in the meshes of a thick capillary network. From the perirenal region of an 11 month old baby.

The figure is drawn from sudan III–stained preparations; objective 5; oil immersion Zeiss microscope.

embryonal life. This type of hemopoiesis has been shown in the adult also, in pathologic states, the outstanding condition being leukemia. Petri [43] has observed small patches of hemopoietic tissue in the retroperitoneal fat in a series of 40 persons, the majority dying of acute infections. Foci of immature blood cells within a fat tissue tumor have been described by Blaisdell,[44] and Babes [45] mentioned normoblasts, megakaryocytes and plasma cells present in a recurrent mesenteric lipoblastoma. In describing a highly cancerous nonlipid retroperitoneal tumor, Warren [46] stressed the presence of foci of immature hematic cells, which he interpreted as originating from embryonal mesenchymal cells of the retroperitoneal fat tissue.

The blood cell-forming potentiality of the undifferentiated connective tissue cells of the fat tissue, as shown in embryonal life and in pathologic states in the adult, might also explain the presence of immature hematic cells in the fat tissue growths in the case under consideration.

### HISTOGENESIS OF SYSTEMIC MULTICENTRIC LIPOBLASTOSIS AND COMPARISON OF THE DEVELOPMENT OF FAT TISSUE IN EMBRYONAL AND IN ADULT LIFE

Kölliker [47] was the first to advance the opinion that the fat cells originate from undifferentiated mesenchymal cells in which fat gradually is deposited. The view of Kölliker has been accepted favorably by a majority; however, discrepancies arose in the identification of the lipogenetic connective tissue cell type. According to Flemming,[48] fat imbibition is a potential function common to all connective tissue cells. Mallory,[10] on the contrary, allocated this property to a specific cell set apart in early life for the sole purpose of storing fat. Further studies by Hammar,[49] Chiari [9] and Inglis [50] show that in regard to embryogenesis there are two sorts of fat tissue cells, both derived from the primitive mesenchyma. In one type the differentiation becomes so well established that an irreversible and specialized tissue located in definite regions of the body is formed—mulberry fat tissue. In the other type the fat tissue cells originate from ordinary connective tissue cells, which temporarily and as an adventitious function have taken over a certain amount of fat, to revert to their previous condition on loss of

43. Petri, E.: Virchows Arch. f. path. Anat. **258**: 37, 1925.
44. Blaisdell, J. L.: Arch. Path. **16**: 643, 1933.
45. Babes, A.: Bull. Assoc. franç. p. l'étude du cancer **18**: 334, 1929.
46. Warren, S.: Am. J. Path. **4**: 51, 1928.
47. Kölliker, A.: Anat. Anz. **1**: 206, 1886.
48. Flemming, W.: Virchows Arch. f. path. Anat. **52**: 568, 1871.
49. Hammar, J. A.: Arch. f. mikr. Anat. **45**: 512, 1895.
50. Inglis, K.: J. Anat. **61**: 452, 1927.

the intracellular fat content. Maximow [51] and Wasserman [40] have more recently cast doubt on this conception. Maximow stated the belief that the specialized fat cell is entirely distinct from the fibroblast of the connective tissue, and as a proof of the high degree of differentiation of the fat cell he stressed its inability to multiply—hence his conclusion that both in embryonal life and in adult life fat tissue cells are formed from undifferentiated mesenchymal cells situated about the blood vessels. The conception of Wasserman is basically the same. He emphasized the interrelationship between the primitive fat organ, the small blood vessels and the "perivascular mesenchymal cells related to the reticulum," to conclude that the adipose tissue is a differentiated part of the reticuloendothelial system. In favor of this conception he mentioned the intimate relationship between hemopoietic tissue and fat tissue in the bone marrow, the formation of hematic cells in adipose tissue and the tendency of the lymphoid tissue to replace fat tissue and conversely (Askanazy [37e] and Bufalini [52]). The presence of argentaffin fibers, identified as pericellular reticulum (Volterra [53]), and the property possessed by the embryonal fat tissue cell and, to a less extent, even by the mature fat cell to store vital dyes (Bremer,[54] Dogliotti [55] and Volterra [56]), lend further support to Wasserman's conclusion.[40] Both Maximow [51] and Wasserman [40] agreed on the point that fat storage in the course of cellular differentiation is accomplished through either local or general metabolic stimuli, the nature of which involves many unsolved problems. Under the influence of these stimuli the undifferentiated mesenchymal cells accumulate fat globules which, as maturation progresses, fuse together to give rise to the swollen spherical cell of the mature fat tissue. Withdrawing of the cytoplasmic processes and flattening and displacing of the nucleus against the cell membrane complete the cellular transformation.

This sequence of events was clearly shown by specimens of adult and embryonic fat tissue which were studied for the purpose of gaining personal acquaintance with the normal development of fat tissue. These observations are summarized briefly so that they may be compared with the findings in the fat tissue growths in the case of systemic multicentric lipoblastosis.

Human embryo 18 cm. long (subcutaneous tissue): Within the primitive connective tissue, irregularly shaped areas were encountered which consisted of young mesenchymal cells and blood capillaries. Evidence of cytoplasmic

51. Maximow, B.: Textbook of Histology, Philadelphia, W. B. Saunders Company, 1934.

52. Bufalini, M.: Arch. ital. di chir. **23**: 281, 1929.

53. Volterra, M.: Sperimentale **81**: 319, 1927.

54. Bremer, J. L.: Anat. Rec. **70**: 263, 1938.

55. Dogliotti, G. C.: Ztschr. f. Zellforsch. u. mikr. Anat. **8**: 222, 1928.

56. Volterra, M.: Sperimentale **77**:242, 1923.

storage of fat globules was noticed in a good number of these cells, suggesting early stages of the process by which mesenchymal cells differentiate into fat cells.

Human embryo 29 cm. long (perirenal region): In the meshes of a loose network of connective tissue fibers foci were encountered which were composed of young mesenchymal cells and blood capillaries. The majority of these cells displayed cytoplasmic processes and large nuclei provided with nucleoli. Within the cytoplasm of some of these cells minute fat droplets were seen, and where the fat droplets were larger and more numerous the cells appeared polygonal or rounded in shape.

Mouse embryo 26 mm. long (region of the thymus): All transitional stages were seen, from fully developed fat lobules, composed of large cells displaying cytoplasmic fat droplets and nucleus either central or displaced to the cell periphery, to small formations, in glandlike arrangement, consisting of cells in more or less advanced stages of fat maturation centered around the walls of branching blood capillaries. These maturing fat cells were irregularly shaped, with large, centrally placed nuclei and various amounts of cytoplasmic fat droplets. Undifferentiated mesenchymal cells with darkly stained nuclei and basophilic cytoplasm were also present.

Baby 2 days old (periadrenal fat tissue): In between mature fat cells, numerous undifferentiated mesenchymal cells were seen, either sparse or in small groups, most numerous around the walls of blood capillaries. Cytoplasmic fat droplets were recognized in some of these cells.

Same baby (region of thymus): In the septums dividing the thymic lobules a fairly complete sequence of progressive stages of fat cell maturation was seen, from undifferentiated mesenchymal cells containing a few cytoplasmic fat droplets to ripe fat cells with nuclei flattened at the periphery.

Baby 11 months old (periadrenal fat tissue): Large polygonal cells with central nuclei and small cytoplasmic fat-globules alternated with mature fat cells showing fusion of fat droplets and nuclei compressed at the periphery. In between these mature or maturing fat cells, undifferentiated mesenchymal cells with dark-stained nuclei and basophilic cytoplasm were seen.

Child 1½ years old (periadrenal fat tissue): In the center of a well developed, mature fat lobule there was a group of polygonal cells with large nuclei, two to three nucleoli and abundant cytoplasm. Within the cytoplasm of these cells small fat globules were recognized.

Rachitic child, age unknown (periadrenal fat tissue): In some areas mature fat cells, with large fat globules and nuclei flattened at the periphery, prevailed. In some other areas polygonal cells with centrally placed, deeply stained nuclei were predominant. Within the latter cells fat droplets of all sizes were seen, and by the confluence of these droplets the ordinary type of fat cell was seen to develop; the nucleus, which was centrally located as long as the individual droplets were discrete, appeared flattened at the periphery on complete maturation of the cell.

White man 42 years old (pericardial fat tissue): The cause of death was phlegmon of the thigh. Within the meshes of a cellular syncytium resulting from the fusion of the cytoplasmic processes of large stellate cells, abundant blood capillaries were encountered. These were surrounded by thin mantles of young mesenchymal cells, which showed distinct cytoplasmic fat droplets. In between these cells mature fat cells were seen. There were concomitant myelocytes, normoblasts and other cell types suggesting extramedullary hematopoiesis.

When the microscopic features found in maturing fat tissue both in embryonal and in adult life under normal conditions are compared with those of the fat tissue overgrowths of the case under study, the fundamentally identical plan of development is apparent, giving further evidence that the nature of the fat tissue overgrowths is more along the line of a hyperplastic process than of a neoplastic one in the strict meaning of the term.

## THE RELATION OF SYSTEMIC MULTICENTRIC LIPOBLASTOSIS TO LIPID STORAGE DISEASES

In recent years much interest has been aroused by a group of disease processes in which large amounts of lipid substances are accumulated in the histiocytes and in the cells of the reticuloendothelial system. It is not clear yet whether this is due to a primary disturbance in the metabolism of lipids which is expressed anatomically by such accumulation of the excessive lipid material carried in the blood stream or whether it is due to a dysfunction of the reticuloendothelial cells leading simultaneously to increased synthesis of lipids and to storing of these within the cells (Pick [57] and Thannhauser [58]).

Further investigations have shown that clinical symptoms and pathologic findings are markedly different from case to case, according to the chemical composition of the accumulated lipids. Although overlapping of pathologic conditions and combinations of forms are not rarely encountered (Sobotka, Epstein and Lichtenstein,[59] Hertzog and colleagues [60] and Epstein [61]), studies along this line have led to the recognition of three main clinical pathologic entities: the cerebroside histiocytosis (Gaucher's disease), the phosphatide histiocytosis (Niemann-Pick disease) and the cholesterol histiocytosis, including Schüller-Christian disease and essential xanthomatosis.

In some of these disease processes—for instance, in Gaucher's disease—reticuloendothelial cells and histiocytes are selectively affected in determinate areas of the body. In other conditions, however—in essential xanthomatosis, for instance—the disease process manifests itself in a diffuse and unpredictable manner, bones, serosal membranes and internal organs being equally affected.

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57. Pick, L.: Am. J. M. Sc. **185**: 453, 1933.

58. Thannhauser, S. J.: Lipoidoses: Diseases of the Cellular Lipid Metabolism, edited by H. A. Christian, New York, Oxford University Press, 1940.

59. Sobotka, H.; Epstein, E. Z., and Lichtenstein, L.: Arch. Path. **10**: 677, 1933.

60. Hertzog, A. J.; Anderson, F. G., and Beebe, G. W.: Arch. Path. **29**: 120, 1940.

61. Epstein, E.: Virchows Arch. f. path. Anat. **298**: 430, 1937.

Histochemical similarities have already suggested an intimate relationship between xanthoma and lipoma (Virchow [62]). Hallopeau [63] and Török [64] have considered the xanthomatous cells as embryonal fat cells, and Waldeyer [65] has shown that the first change in xanthoma consists in the appearance of fatty globules in perivascular mesenchymal cells, the same as that which occurs in the lipoblastic growths. Association in the same person of lipoma and xanthoma (Ehrmann [66]) also points to an intimate relationship between the two processes.

Chemical investigations by Bonnefous and Valdiguié [67] showed high blood cholesterol in cases of diffuse lipomatosis with entirely subcutaneous distribution. Traina Rao,[68] in 10 cases of lipoma, found excessive lipids in the blood, including cholesterol, neutral fats, soap and phosphatides. Goormaghtigh and associates [21] demonstrated that the mature fat cells of lipoma contain lecithin and cholesterol while the embryonal fat cells of lipoma contain neutral fat and fatty acids.

Since biochemical investigations were not made in our case or in the 6 similar cases in the literature, one can only wonder whether diffuse multicentric lipoblastosis also is due to altered metabolism of the lipid system, with incidental stimulation of undifferentiated mesenchymal cells leading to lipid storage, the whole resulting in tumor-like growths in multicentric areas.

## SUMMARY

Multiple, recurrent nonencapsulated fat tissue growth, involving in a scattered and disorderly fashion both the subcutaneous tissue and the internal cavities of the body, was the outstanding characteristic of the case reported here. The process started in the popliteal space as an apparently simple lipoma, which was removed; the multicentric proliferation of fat tissue began six years later, and in spite of the numerous attempts to control the process by surgical intervention, a series of recurrences brought the patient to death in the course of twelve years.

Only 6 cases which might be compared with the one now reported were found in the literature, the majority under inconspicuous or misleading labels, such as "multiple lipoma," "lipoblastic sarcoma with metastasis," "metastasizing lipoma" or "lipoblastic sarcoma."

Distinctive characteristics emerging from the study of all these cases seem to point to a separate entity in the broad class of the lipid system overgrowths. The label "systemic multicentric lipoblastosis," proposed by Goormaghtigh,[21] might be appropriate for this.

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62. Virchow, R.: Virchows Arch. f. path. Anat. **52**: 504, 1871.
63. Hallopeau: Ann. de dermat. et syph. **4**: 595, 1903.
64. Török: Ann. de dermat. et syph. **4**: 1109 and 1261, 1893.
65. Waldeyer, W.: Virchows Arch. f. path. Anat. **52**: 318, 1871.
66. Ehrmann, cited by Ewing.[20]
67. Bonnefous, R., and Valdiguié, A.: Ann. de dermat. et syph. **5**: 290, 1924.
68. Traina Rao, G.: Riv. ital. di ginec. **19**: 1, 1936.

Among the clinical-pathologic features common to these cases, the following are the most consistent: (1) long duration of the disease process; (2) independent nonencapsulated fat tissue growths involving in an unpredictable and disorderly fashion subcutaneous tissue, internal cavities, bones and deep organs; (3) striking tendency of the fat tissue growths to recur after excision; (4) predominance in tissue of mature fat cells, with frequent transitional forms indicating that they originate from undifferentiated mesenchymal cells according to a plan which does not differ fundamentally from that by which fat tissue develops in either embryonal or adult life under normal conditions, and (5) lack of evidence of cellular anarchy and absence of mitotic figures and of any other cellular or structural pattern suggesting a neoplastic growth.

These being the main characteristics of the process, it seems unlikely that these cases belong in the category of cases of fat tissue tumors in the strict meaning of the term. Both for the case presented here and for the similar ones in the literature the possibility is conceived that the basic manifestation of the process is an alteration of the metabolism of the lipid system, with incidental stimulation of undifferentiated mesenchymal cells leading to lipid storage, the whole resulting in tumor-like growths in multicentric areas.

# Case Reports

## PRIMARY HODGKIN'S SARCOMA OF THE BRAIN

H. J. SPARLING Jr., MD., and R. D. ADAMS, M.D., BOSTON

SECONDARY lymphomatous involvement of the nervous system, though relatively infrequent, is well recognized. This subject has been adequately presented and thoroughly reviewed by Verda,[1] Browder and de Veer,[2] Ginsberg,[3] Blakeslee,[4] Von Hagen,[5] Winkelman and Moore,[6] Gray, Baker, Cottrell and Skogland[7] and Jackson and Parker.[8] In most of the cases reported by these authors the tumor metastasized to the brain by way of the blood stream, or, having invaded a skull bone, a vertebra or the spinal epidural space, involved the nervous tissue by direct extension or by compression. There are several cases in which the initial symptoms were referable to the nervous system, and the only clinically demonstrable tumor was in the spinal epidural space, the neoplasm having arisen there or having extended there from the vertebrae or from paravertebral tissues through the intervertebral foramens.

The possibility of a lymphoma arising in the substance of the brain and spinal cord has been hypothecated, but only a few examples of this have been recorded. Yuile[9] and Kinney and Adams[10] have reported 3 cases of primary reticulum cell sarcoma of the brain. The latter authors collected from the medical literature 5 similar cases which had been reported under the names "perithelial sarcoma," "microglioma" and "microglioblastoma." To date there have been no reported cases of Hodgkin's sarcoma, granuloma, or paragranuloma or of giant follicle lymphoma originating within the nervous system. Two cases of primary lymphosarcoma of the brain have been described by Abbott and

From the Mallory Institute of Pathology and the Neurological Unit, Boston City Hospital, and the Department of Neurology, Harvard Medical School.

1. Verda, D. J.: M. Clin. North America **24**:1228, 1944.
2. Browder, J., and de Veer, J. A.: Arch. Neurol. & Psychiat. **41**:328, 1939.
3. Ginsberg, S.: Arch. Int. Med. **39**:571, 1927.
4. Blakeslee, G. A.: Arch Neurol. & Psychiat. **20**:130, 1928.
5. Von Hagen, K. O.: Bull. Los Angeles Neurol. Soc. **20**:20, 1937.
6. Winkelman, N. W., and Moore, M. T.: Arch. Neurol. & Psychiat. **45**: 304, 1941.
7. Gray, R. C.; Baker, A. B.; Cottrell, L., and Skogland, J. E.: Clinics **4**: 230, 1941.
8. Jackson, H., Jr., and Parker, F., Jr.: New England J. Med. **233**:369, 1945.
9. Yuile, C. L.: Arch. Path. **26**:1086, 1938.
10. Kinney, T. D., and Adams, R. D.: Arch. Neurol. & Psychiat. **50**:552, 1943.

Adson.[11] In these cases the tumor appeared to have originated in the cerebral meninges, but it was impossible to exclude a skull bone as the primary source. With the permission of Dr. J. W. Kernohan, we have examined microscopic sections in these 2 cases. The second case was unquestionably one of lymphosarcoma; the tumor in the first case, in our opinion, conforms in all respects to reticulum cell sarcoma. Unfortunately, a postmortem examination was not made in the case of lymphosarcoma. Therefore we must conclude that primary lymphosarcoma of the brain is not as yet an established pathologic entity.

In the past year at the Mallory Institute of Pathology a complete autopsy has been done in a case of primary Hodgkin's sarcoma of the brain. The patient had been admitted to the neurologic wards of the Boston City Hospital with symptoms of a tumor of the left frontal lobe. We are prompted to report this case because no report of one like it has appeared in medical literature.

## REPORT OF A CASE

Approximately three months prior to entering the hospital the patient, a 53 year old white man, began to be drowsy and confused. These symptoms, at first intermittent, became more pronounced and persistent and were succeeded by a disturbance of speech, namely, slowness and difficulty in finding the correct words. Six weeks later he collapsed while at work. At this time he had a severe headache and an unsteady gait. Weakness of the right arm and leg developed soon afterward. Several times at night his wife noted jerking movements of his arms and legs, which were probably convulsions. Increasing confusion and nausea and vomiting led to his entering the hospital.

At the time of his admission the temperature, the pulse rate, the respiratory rate and the blood pressure were all within normal limits. The patient was drowsy and unable to give a satisfactory history. He was inattentive and forgetful and responded slowly and inadequately to all stimuli. There was spastic right hemiparesis affecting the face, the arm and the leg. The tendon reflexes of the right side were more active than those of the left; the right abdominal reflexes were absent; the right plantar reflex was extensor, whereas the left was flexor. The visual field and the optic fundi were normal, and there was no impairment of cutaneous sensation. The liver was enlarged, the lower edge being 7 to 8 cm. below the costal margin.

The hemoglobin concentration was 80 to 90 per cent of normal. The white blood cell count and the differential count were within the limits of normal. A determination of the blood sugar showed 180 mg. per hundred cubic centimeters during fasting. The Hinton test of the blood revealed no syphilis. The cerebrospinal fluid was under normal pressure and contained 10 lymphocytes per cubic millimeter and 333 mg. of protein per hundred cubic centimeters; the colloidal gold curve was 5555443321, and the Wassermann and Davies-Hinton tests of the fluid were negative. No abnormalities were seen in roentgenograms of the skull, the spine, the chest, the pelvis and the proximal portions of the long bones. In an electroencephalogram taken on the ninth hospital day there were six to eight waves per second in all leads and marked asymmetry between the two

11. Abbott, K. H., and Adson, A. W.: Arch. Surg. **47**:147, 1943.

hemispheres, especially between the right and the left frontal lobe. The lower voltage and the less regular activity were over the left frontal lobe.

During the first eight days in the hospital the patient's condition became much worse. He was more confused and was unable to find correct words in attempts to express himself or to comprehend spoken words clearly. The right arm and leg became weaker and more spastic, and there was impairment of pain and of touch sensation over the right side of the body. He was incontinent of urine and feces.

On the tenth hospital day a ventriculogram was made, which disclosed that the lateral and third ventricles were slightly displaced to the right and that the anterior horn of the left lateral ventricle was obliterated. After this procedure the patient became comatose, and two days later craniotomy was done. The dura was found to be tense, and when it was reflected, there was exposed a firm, grayish white nodule in the superior frontal convolution, approximately 2 cm. from the frontal pole. The anterior portion of the left frontal lobe was resected. After operation the patient remained in coma. Gradually the body temperature rose, the blood pressure fell and the breathing became stertorous; the patient died on the fifth postoperative day.

*Surgical Specimen.*—The excised tissue showed a firm, grayish white mass, measuring 2 by 1.5 cm., embedded in edematous brain tissue. On the cut surface the mass was well demarcated from the surrounding brain tissue and was flecked with minute red and yellow areas.

Tissue from the tumor was fixed in Zenker's fluid and in solution of formaldehyde U. S. P. diluted 1 to 10 and was stained with phloxine–methylene blue, Foot's modification of Hortega's stain for reticulum and Mallory's phosphotungstic acid–hematoxylin and aniline blue stains for collagen.

In microscopic sections large masses of tumor cells replaced the cerebral cortex and white matter. Under low magnification the tumor presented many well preserved areas separated by broad bands of necrotic tissue. The viable tumor consisted of sheets of cells approximately 15 microns in diameter, without syncytial relations and forming loosely arranged groups and clusters. Viable cells tended to be grouped around blood vessels. The nucleus of the tumor cell was large and round to ovoid and contained moderately coarse, evenly dispersed particles of chromatin; from one to three prominent nucleoli were visible; the cytoplasm was relatively sparse and palely basophilic. Single lobulated, binucleated and multinucleated giant cells were abundant (fig. 1). Numerous mitotic figures were present. No glial fibrils were seen. There was an abundance of reticulum in the form of a network of fibers which surrounded single cells and small clusters of cells (fig. 2). Sparsely scattered collagen fibers were found throughout the tumor.

*Autopsy* (two and one-half hours after death).—When the incision of the scalp was opened, a small amount of malodorous grayish purulent material was exposed. This exudate had collected on the external surface of the dura and to a slight extent in the subdural space. The leptomeninges were opaque, and purulent exudate was seen in the subarachnoid space. The brain weighed 1,350 Gm. There was a large defect in the left frontal lobe. The adjacent brain tissue was soft, granular and hemorrhagic. No tumor tissue could be identified. The choroid plexuses were covered with purulent exudate, and there were numerous petechial hemorrhages beneath the ependyma of the posterior horns of the lateral ventricles. The dural sinuses, the bones of the base of the skull and the paranasal sinuses revealed no evidence of tumor.

There was fibrous pleuritis on the right side. The liver weighed 2,200 Gm. Its surface was pale and presented nodules varying up to 2 mm. in diameter. It sectioned with increased resistance, and on the cut surface there were similar yellow nodules separated by very thin gray fibrous bands. There were no

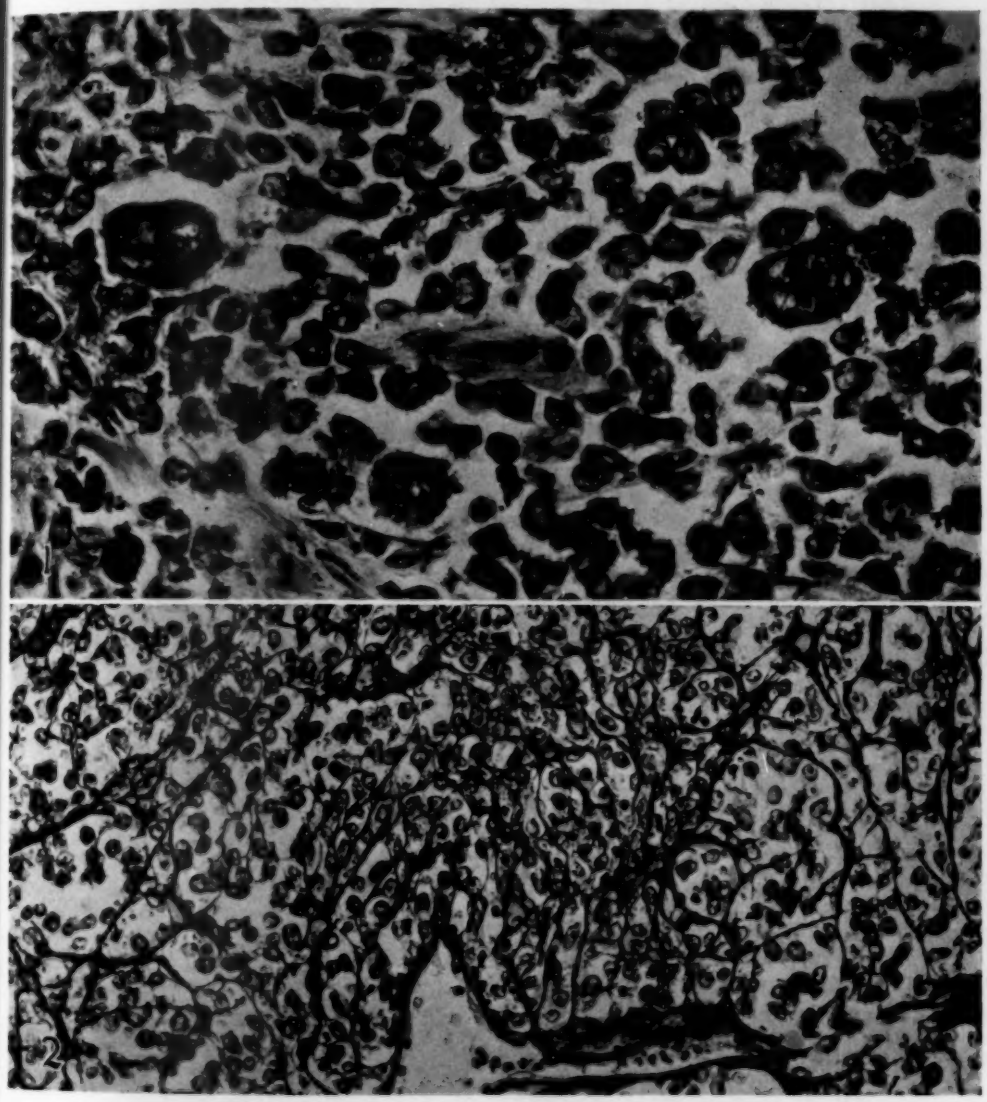

Fig. 1.—Section of tumor showing characteristics of sarcomatous growth, tumor cells with large nuclei and prominent nucleoli, and three multinucleated Reed-Sternberg giant cells. Phloxine-methylene blue; × 780.

Fig. 2.—Section of tumor showing reticulum fibers distributed between groups of cells and individual cells. Foot's modification of the Hortega stain for reticulum; × 280.

enlarged lymph nodes. Several small abscesses were found within the substance of the prostate gland. Postmortem roentgenograms of the bones of the extremities revealed no abnormalities.

*Bacteriologic Report.*—Escherichia coli and hemolytic Staphylococcus aureus were isolated from the exudate over the dura, and E. coli and Bacillus proteus from the subarachnoid space. A few colonies of Staph. aureus were obtained on culture of the heart's blood. In cultures of the lung there were a few colonies of B. proteus and hemolytic Staph. aureus. Similar organisms were obtained from the prostate gland.

*Microscopic Examination.*—Only the brain and the liver were of significant interest. The exudate on the outer surface of the dura contained fibrin, many polymorphonuclear leukocytes and bacteria, both rods and cocci. A similar exudate was attached to the inner surface of the dura. The cerebral and spinal leptomeninges were infiltrated by large numbers of polymorphonuclear leukocytes and a few lymphocytes, and a similar exudate had collected in the subarachnoid space. No tumor cells were present in either the brain or the meninges. In sections of the brain tissue adjacent to the excised area there was widespread necrosis of nerve and glial cells, with many petechial hemorrhages, as well as an infiltrate of polymorphonuclear leukocytes.

The normal structure of the liver was distorted by thin fibrous septums, the presence of which resulted in the formation of small nodules. The increased fibrous tissue was primarily portal in distribution. Many hepatic cells contained large or small vacuoles. Alcoholic hyalin was not identified.

The remaining histologic sections served only to confirm the other gross findings, which were of minor importance.

*Anatomic Diagnosis.*—(1) Primary Hodgkin's sarcoma of the left frontal lobe of the brain; (2) acute purulent pachymeningitis, leptomeningitis and localized bacterial encephalitis, postoperative; (3) alcoholic cirrhosis of the liver; (4) pulmonary atelectasis, emphysema and congestion, and (5) acute prostatitis with abscess formation.

## COMMENT

The principal symptoms in this case were confusion, partial motor aphasia and right hemiparesis. They clearly indicated extensive damage of the left frontal lobe. In view of the age of the patient and the fairly rapid and progressive evolution of the symptoms it was obvious that there was a rapidly growing tumor of the brain even though the cerebrospinal fluid pressure was normal. There was no sign of carcinoma of the lungs or other viscera; hence the clinical diagnosis was glioblastoma multiforme. The terminal elevation of temperature and the persistent coma were probably related to the infection of the wound and the acute bacterial leptomeningitis.

The tumor tissue had the usual gross appearance of Hodgkin's sarcoma. It was firm, homogeneous and in places flecked with red and yellow areas. Histologically the tumor was characteristic of Hodgkin's sarcoma. The cell type was a large mononuclear cell with a large nucleus and prominent nucleoli. Reed-Sternberg giant cells were abundant, and the reticulum formed a delicate intercellular stroma.

That the brain was the site of origin seems indisputable. There was no clinical evidence of involvement of cervical lymph nodes, of paranasal sinuses or the nasopharynx, of other viscera or of bones. Finally, postmortem roentgenograms of the long bones and a complete autopsy failed to show tumor in other organs from which a cerebral metastasis could have arisen.

Although histologically separable, Hodgkin's sarcoma and reticulum cell sarcoma often have a similar clinical course. It is of interest in this respect to compare our case with the 2 cases of primary reticulum cell sarcoma of the brain reported by Kinney and Adams [10] and the 5 cases which they collected from the literature. All the patients with reticulum cell sarcoma were males, varying in age from 9 years to 72 years, with an average age of 44 years. The average duration of symptoms prior to operation was approximately six months, and the period of survival after the operation averaged three months. In all these patients the tumor was situated in the temporal lobe. Similarly, our patient with Hodgkin's sarcoma was old, and the clinical course was brief (three months). In contrast to the reticulum cell sarcoma, which in all 7 cases was located in the temporal lobe, the tumor in our case was situated in the frontal lobe.

The precise cell type of Hodgkin's sarcoma is not definitely known. Some authorities believe that both reticulum cell sarcoma and Hodgkin's sarcoma are derived from the reticulum cell or the histiocyte and differ from each other only in minor details. In other words, these tumors are essentially two forms of a histiocytic sarcoma. If this assumption is correct, Hodgkin's sarcoma of the brain would be expected to arise from histiocytic cells of the meninges or the adventitia of the blood vessels. A third hypothetic source would be the microglial cells, which, according to the studies of Hortega,[12] are derived during fetal life from histiocytes at certain fixed points in the leptomeninges. It is not intended in the foregoing discussion to suggest that the pathologic distinction between reticulum cell sarcoma and Hodgkin's sarcoma should be abandoned. The occurrence of primary reticulum cell sarcoma of bone with its relatively good prognosis, contrasted with the absence of primary involvement of bone in Hodgkin's sarcoma, and the not infrequent transition of Hodgkin's granuloma to Hodgkin's sarcoma but never to reticulum cell sarcoma make a distinction between these two tumors important.

In recent years 2 cases of cerebellar involvement by a malignant tumor with the histologic characteristics of Hodgkin's sarcoma have been seen at the laboratory with which we are associated. One of

12. del Rio Hortega, in Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932.

these cases has been previously reported in detail.[8] In both, the short duration of the clinical course and the initial signs and symptoms of a rapidly growing intracranial malignant neoplasm were similar to the case reported here. Careful clinical study detected no involvement of lymph nodes, nasopharynx, viscera or skeleton by a malignant tumor which might have given rise to a cerebral metastasis. Necropsy showed a malignant tumor of the cerebellum with the characteristic microscopic appearance of Hodgkin's sarcoma. Unfortunately, only the brain was examined in each instance. Although the clinical evidence indicates that these 2 cases were examples of primary Hodgkin's sarcoma of the cerebellum, a definite conclusion cannot be drawn because of the lack of complete pathologic confirmation.

SUMMARY

To the best of our knowledge the case of Hodgkin's sarcoma of the left frontal lobe of the brain which we have described is the first case of primary Hodgkin's sarcoma of the brain to be reported. In addition, 2 cases of Hodgkin's sarcoma of the cerebellum have been encountered, but the proof of the site of origin is not conclusive since only the brain was submitted for pathologic examination.

# PRIMARY MELANOMA OF THE ADRENAL GLAND

RALPH M. KNISELEY, M.D.
Fellow in Pathology, Mayo Foundation
and
ARCHIE H. BAGGENSTOSS, M.D.
ROCHESTER, MINN.

PRIMARY melanoma of the adrenal gland is very rare. Only a few reports of melanoma that can be considered of true adrenal origin are to be found in the literature. As Goldzieher [1] pointed out, the diagnosis requires great caution because of the possibility that a small unrecognized primary tumor of the eye or the skin is present with predominant secondary involvement of the adrenal gland. The histogenesis of such a primary growth has interested many workers and will be discussed later in this paper.

## LITERATURE

In 1914 Pappenheimer [2] reported a case of primary melanoma of the adrenal gland. At that time he accepted only 5 cases, which had included references to the eyes, as adequately reported. These were the cases of Schweikert, Davidsohn (case 2), Schumm and Molnar and his own case. Goldzieher considered as authentic the cases of Davidsohn, Schumm, Neuberg, Goldzieher, Pappenheimer, Schmidt and Maclachlan. The case reported by MacLachlan [3] in 1915 was that of a bilateral melanotic growth with extensive metastasis. In 1927 R. M. Smith [4] described a primary melanotic growth of the adrenal glands. In 1933 McComb and D. B. Smith [5] reported a case of primary bilateral malignant tumor of the adrenal glands with secondary melanotic growths in the subcutaneous tissue and the urinary bladder. The primary neoplasm did not contain melanin. In 1938 Baker [6] described a pigmented adenoma of the adrenal gland. The pigment reacted to various reagents as melanin does, but the tumor did not have the characteristic gross or microscopic structure of melanoma, nor was there evidence of tumor elsewhere. It is fairly certain that there are not more than 11 authentic cases reported. Recently we encountered a case of primary cancerous melanoma of the adrenal gland.

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From the Section on Pathologic Anatomy, Mayo Clinic.

1. Goldzieher, M. A.: The Adrenal Glands in Health and Disease, Philadelphia, F. A. Davis Company, 1944, p. 104.
2. Pappenheimer, A. M.: Proc. New York Path. Soc. **14**:173, 1914.
3. MacLachlan, W. W. G.: J. M. Research **33**:93, 1915.
4. Smith, R. M.: M. J. Australia **1**:683, 1927.
5. McComb, R. A., and Smith, D. B.: J. Urol. **30**:49, 1933.
6. Baker, M. R.: Arch. Path. **26**:845, 1938.

REPORT OF A CASE

The patient was a 60 year old white man who registered at the Mayo Clinic first on July 12, 1944 with symptoms of duodenal ulcer which had been diagnosed and treated elsewhere for two years. He returned July 26, 1945, with intermittent pain over the lower part of the back which extended down the thighs. He complained of anorexia and had lost 40 pounds (18.1 Kg.) during the previous year.

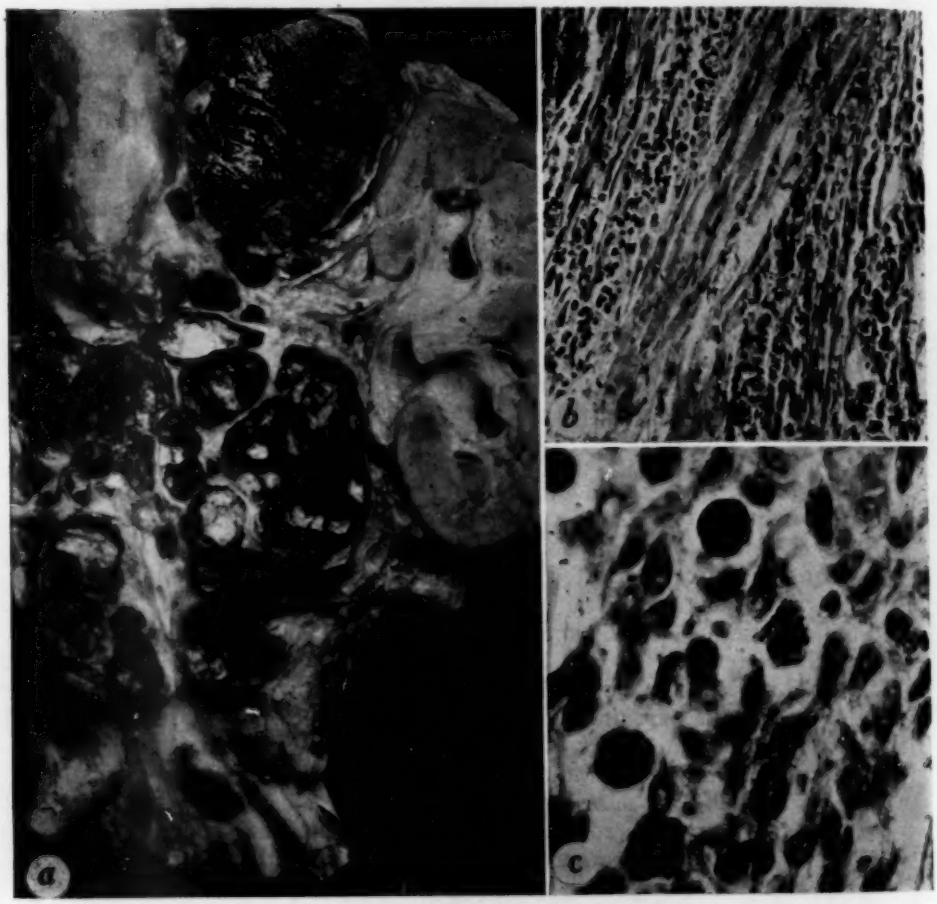


Fig. 1.—Melanoma of the left adrenal gland: (*a*) Gross view of the growth with metastases in lymph nodes. (*b*) Histologic structure of the primary neoplasm (x 145). Compressed cortex is visible. (*c*) Structure of the cancerous cells (x 475). The melanin pigment is seen in a number of these cells.

The essential finding at examination was a palpable, questionably retroperitoneal mass in the midabdomen. Tenderness over the fourth lumbar vertebra was present. The roentgenograms revealed destruction of the fourth lumbar vertebra. Roentgen therapy was given to the lumbar region, with relief of pain. On September 12, coma ensued with auricular fibrillation, cyanosis and a fall of blood pressure to 50 mm. of mercury systolic and 40 diastolic. Death occurred on September 12.

*Necropsy.*—The body was moderately emaciated. A tumor mass measuring 8.5 by 5.5 by 4.5 cm. was present at the superior pole of the left kidney (fig. 1*a*). It was brownish black and soft, though not friable. It appeared to have originated from and destroyed the left adrenal gland. Numerous pigmented tumor masses surrounded the superior, medial and inferior borders of the left kidney. Some of these masses showed central necrosis. Five centimeters above the point where the left ureter entered the bladder, pigmented tumor nodules obstructed the lumen. Above this obstruction, the ureter and the renal pelvis were moderately dilated. Infected hydronephrosis with abscess of the left kidney was present. The right adrenal gland contained a small pigmented tumor nodule and was moderately atrophied.

The skin showed no pigmented growths. The brain and the spinal cord and their meninges were normal. The choroid and the retina of each eye were removed, examined closely and found to be normal.

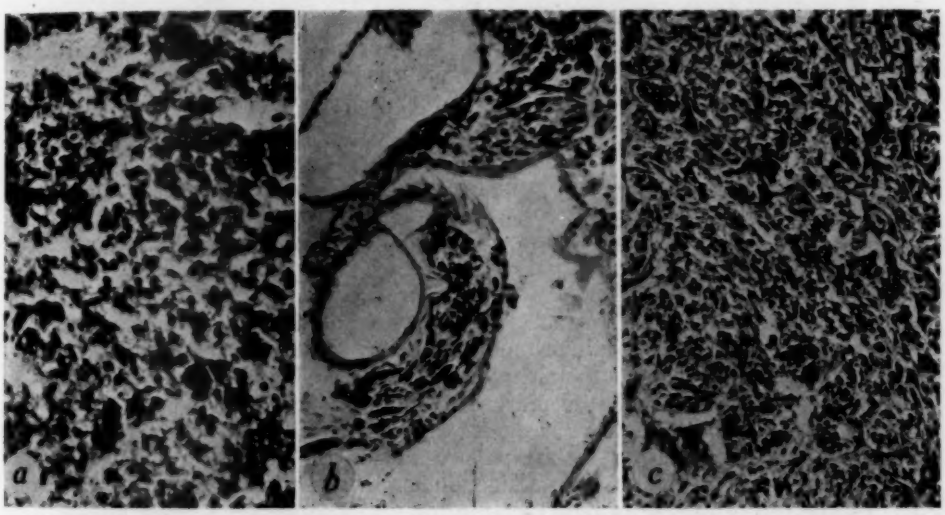


Fig. 2.—Metastatic lesions: (*a*) Metastatic neoplasm of the right adrenal gland treated with Becker's silver nitrate reagent. Note the reduction of silver nitrate (x 200). (*b*) Pigmented metastasis in the lung (x 140). (*c*) Metastatic neoplasm in the liver. The cells are similar to the neoplastic cells elsewhere, but no pigment is present (x 95).

The superior mediastinal lymph nodes were enlarged and contained melanotic tumor masses. In the liver there were smooth, yellowish white, sharply circumscribed tumor nodules, measuring 1.0 to 3.0 cm. in diameter. They were present both on the surface and in the substance of the liver. The intestinal tract showed no visible abnormalities, but lymph nodes in the region of the head of the pancreas were involved with pigmented metastatic growths. Pigmented metastatic growths were present in the bodies of the twelfth thoracic and the first, second, third, fourth and fifth lumbar vertebrae. Scarring of the duodenum 1 cm. distal to the pylorus was observed and interpreted as evidence of a healed duodenal ulcer. Edema of the lungs and hydrothorax (250 cc. on the left and 100 cc. on the right) also were present.

*Histologic Examination.*—The primary tumor was composed of sheets of spindle-shaped cells with many regions of necrosis (fig. 1*b*). The tumor cell (fig. 1*c*) had a prominent oval to spindle-shaped nucleus with a reticular network and pink granular cytoplasm with an indistinct border. Mitotic figures were numerous, and many of the nucleoli were large and prominent. Large numbers of cells contained coarse, golden brown pigment granules in the cytoplasm. In many areas the pigment was so abundant that cellular details could not be seen. The berlin blue reaction for iron with a counter stain of basic fuchsin was negative; however, the pigment was blackened when Becker's silver nitrate solution was used as reagent. The dark color of melanin is brought out by oxidizing agents. Mucus was not present in sections treated with the Dresbach [7] modification of Meyer's mucin stain. No production of reticulum was seen in tissue stained by the Gomori reticulum method. An associated acute inflammatory reaction appeared in the scattered necrotic areas.

The metastatic tumors in the regional nodes, the right adrenal gland, the periureteral region and vertebrae which were noted grossly corresponded microscopically to the histologic picture described in the preceding paragraph (fig. 2*a*). Metastatic lesions also were detected in the lungs (fig. 2*b*) and the prostate on histologic examination of these organs. The nodules in the liver (fig. 2*c*) were made up of cells which resembled the ones described, but no production of pigment was observed.

## COMMENT

Because of the rarity of primary melanoma of the adrenal gland, a thorough search was made for a commoner primary source such as the eye or the skin. There was no primary neoplasm in the skin, nor was there a history of the removal of a mole. There were no ocular symptoms, and, as noted previously, the choroid coats were examined carefully and found to be normal. The rectum and the anus were free of tumors, as were the meninges and the central nervous system. Because of the absence of any other identifiable origin and because of the large size of the tumor in the left adrenal gland, with its local nodal involvement and distant metastatic lesions, we felt justified in considering this as a case of cancerous melanoma primary in the adrenal gland. The tumor was identified to our satisfaction as a melanin-producing neoplasm by the use of the berlin blue reaction for iron and Becker's silver nitrate reagent. These were deemed to give adequate evidence of the nature of the pigment, since Baker has shown that only these two reagents give any valuable histologic information regarding melanin-like substances. Bloch's "dopa" was not available, and since the necropsy was performed after vascular embalming, the reaction with this reagent would have been of no aid in identification.

As has been true of other investigators of the subject, we have had our curiosity aroused regarding the histogenesis of tumors of this type. Conflicting views have been presented in the literature. Most of those who have worked on the problem agree that epinephrine is produced in the adrenal medulla and that a close chemical relation exists between epinephrine and melanin. Epinephrine has been oxidized to a melanin-like pigment by a variety of methods. Edlbacher [8] cited the work of

7. Dresbach, M.: Personal communication to the authors.

8. Edlbacher, S., in Luck, J. M.: Annual Review of Biochemistry, Stanford University, Calif., Stanford University Press, 1937, vol. 6, pp. 275-277.

Schuler and his associates, who concluded that tyrosine is the mother substance of epinephrine. Tyrosine when oxidized by the enzyme tyrosinase yields melanin pigment. It is therefore natural to postulate that the cells of the adrenal medulla are the source of the melanoma, since these cells are the producers of epinephrine. Kaufmann [9] referred to groups of melanotic pigmented ganglion cells in the medulla arising from the sympathetic formative cells. These could conceivably serve as the predecessors of the tumor. In support of this concept, Millar [10] has reported a cancerous melanotic tumor of the ganglion cells, arising from a thoracic ganglion. McComb and Smith offered the pheochromocyte as the possible cell of origin of the neoplasm in their case of melanotic tumor. MacLachlan, however, was not convinced of this source, because of the appearance of the cells of the tumor that he reported. He has advocated the chromatophore, which is found in the loose connective tissue about the adrenal gland as the source. Goldzieher was impressed with the similarity of structure between the melanotic and the unpigmented cortical tumors, and expressed the belief that the cells of the cortex are the source of these tumors. He also pointed out the fact that benign adenoma of the cortex is by no means rare.

We have occasionally observed small, brownish black nodules, so-called melanomas, in the adrenal cortex and wondered whether one of these might possibly have been the source of the neoplasm described. An investigation of a number of these nodules in 21 other cases was carried out. The pigment was found to have some of the properties of a lipoid, but no melanin could be identified. This led us to abandon the idea that these small pigmented cortical tumors might be the predecessors of cancerous melanoma. It would seem that the precise cell of origin in cases of cancerous melanoma of the adrenal gland is still in doubt.

SUMMARY

Various sources for the cell of origin of cancerous melanoma of the adrenal gland have been postulated but our observations in a case of primary cancerous melanoma of the left adrenal gland with widespread metastatic growths did not allow a conclusion as to the histogenesis of the tumor.

9. Kaufmann, E.: Pathology for Students and Practitioners, translated by S. P. Reimann, Philadelphia, P. Blakiston's Son & Co., 1929, vol. 2.

10. Millar, W. G.: J. Path. & Bact. **35**:351, 1932.

# Laboratory Methods and Technical Notes

## A CENTRIFUGE METHOD OF DETERMINING BLOOD PROTEINS

JULIAN H. LEWIS, Ph.D., M.D., CHICAGO

SEVERAL methods have been described for the estimation of the protein of urine and cerebrospinal fluid in which the volume of precipitated protein is measured after the urine or the fluid has been centrifuged in special graduated tubes (Shevky and Stafford [1]; Young and Bennett [2]; Young, Bennett, Christlieb and Myers [3]; McNaught [4]; Bauer and Schenck.[5]) Experiments have been carried out which show that the principle of these methods can be used in the estimation of the total protein, the albumin and the globulin of blood serum. Shevky and Stafford suggested that their method be used for estimating the protein of serum and other body fluids but gave results of its use only with urine. No way of standardizing the procedure other than timing the period of centrifugation has been given. These investigators claim only approximately accurate results. A calibrated centrifuge tube was used by them, but they gave no description of it. Peters and Van Slyke [6] pictured a graduated tube described in a personal communication from McKay, whose results for the protein of urine when he used this tube in a modification of the Shevky and Stafford method were accurate to within 10 per cent.

Of the special centrifuge tubes described, that of Bauer and Schenck appears to be the one best adapted for use in measuring the volume of precipitated serum protein. Its construction is such that there is small likelihood of the precipitate being impacted in the shoulder of the tube and thus prevented from entering the tip. The long, narrow, tapered tip holds 0.4 cc. and is graduated to 0.004 cc. Estimations of 0.002 cc. can be easily made. The accuracy of the graduations of tubes obtained at a local supply house has been checked and found satisfactory. A minor disadvantage is their total capacity of only 3 cc.

Comparative experiments have shown that the tungstic acid reagent of Folin and Wu is the most suitable precipitant. It produces a finely granular soft precipitate that packs evenly on being centrifuged. Its

From the Provident Hospital.

This investigation was aided by a grant from the Otho S. A. Sprague Memorial Institute for Medical Research.

1. Shevky, M. C., and Stafford, D. D.: Arch. Int. Med. **32**:222, 1923.

2. Young, G. A., and Bennett, A. E.: Am. J. M. Sc. **172**:249, 1926.

3. Young, G. A.; Bennett, A. E.; Christlieb, J. M., and Myers, J. T.: Arch. Neurol. & Psychiat. **23**:542, 1930.

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5. Bauer, A. R., and Schenck, H. P.: J. Lab. & Clin. Med. **16**:1090, 1931.

6. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, Williams & Wilkins Company, 1932, vol. 2.

further advantage is that it is the precipitant usually employed in the chemical estimation of serum protein, this being the method by which the centrifuge method is standardized.

## PROCEDURE

The precipitant is prepared by mixing 1 part of 10 per cent sodium tungstate, 6 parts of distilled water and 1 part of two thirds–normal sulfuric acid. This mixture can be kept for two weeks, after which it must be discarded and a fresh one prepared (Van Slyke and Hawkins [7]). One and eight-tenths cubic centimeters of the solution is measured into the Bauer-Schenck tube. It is best that the precipitant be not allowed to enter the narrow tip of the tube, in order to facilitate future mixing. With a small bore accurate pipet 0.2 cc. of clear serum is now measured into the tube. The mixture is stirred well with a thin glass rod, the stirrer making certain that none of the precipitate adheres to the glass walls of the tube. One more cubic centimeter of the precipitant is added, which is used to wash the glass rod and bring the volume to 3 cc. A sharp tapping of the tube fills the tip and further mixes the contents. The tube is capped with a sheet of

TABLE 1.—*Volumes of Protein Precipitated from Serum Treated by the Centrifuge Method*

| Samples of Serum with 7.1 per Cent Protein, Cc. | Percentage of Protein If Sample Were Contained in 0.2 Cc. | Volume of Precipitate, Cc. |
|---|---|---|
| 0.22 | 7.81 | 0.336 |
| 0.20 | 7.10 | 0.306 |
| 0.18 | 6.39 | 0.276 |
| 0.16 | 5.68 | 0.246 |
| 0.14 | 4.97 | 0.216 |
| 0.12 | 4.26 | 0.186 |
| 0.10 | 3.55 | 0.156 |
| 0.08 | 2.84 | 0.126 |

cellophane held in place by a rubber band. The tube is then centrifuged for fifteen minutes at full speed, the centrifuge being started as rapidly as can be safely done. With the aid of a magnifying glass, the volume of the precipitate is read.

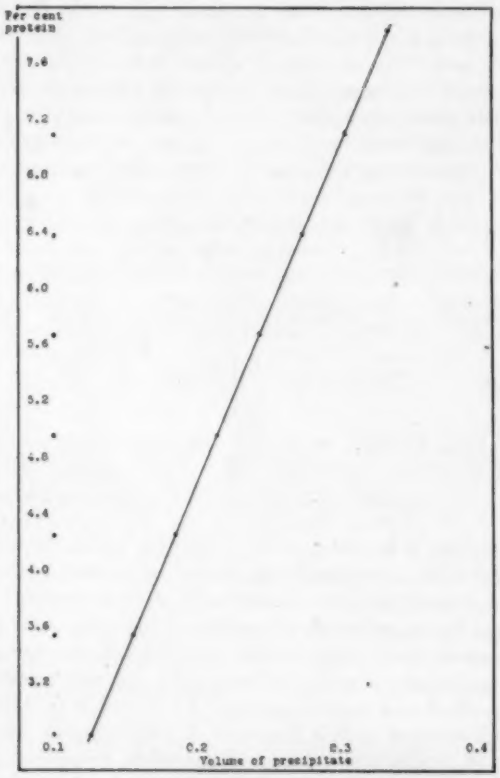
In order to find the quantitative relationship of the volumes of various amounts of protein precipitated from serum treated according to the procedure described in the foregoing paragraph, a serum containg a 7.1 per cent protein as determined by the Kjeldahl method was used in amounts of 0.22, 0.2, 0.18, 0.16, 0.14, 0.12, 0.10 and 0.08 cc. The volume of protein contained in these samples corresponds to that in 0.2 cc. amounts of serums whose protein contents are, respectively, 7.81, 7.10, 6.39, 5.68, 4.97, 4.26, 3.55 and 2.84 per cent. The volumes of protein obtained are given in table 1. When these volumes are plotted against the percentages of protein, a straight line results (chart).

It will be noted that the volume of precipitate is large relative to the volume of the sample used. This is due to the high hydration of the precipitated protein. By prolonged centrifugation the water content can be materially reduced. A series of precipitates similar to the ones obtained in this experiment were centrifuged for one hour and thirty minutes at full speed with interruptions at fifteen minute intervals for readings. These showed that the volume is reduced rapidly during the first fifteen minute periods and that although reduction was progressively slower during each succeeding period there was still a significant reduction during the

7. Van Slyke, D. D., and Hawkins, J. A.: J. Biol. Chem. **79**:739, 1928.

sixth and final period. However, the volumes of the precipitates at each reading when plotted against percentages of protein formed a straight line curve. These lines changed in slope during the first three periods but were parallel during the remaining periods.

Many experiments have shown that the centrifugal force developed by a centrifuge cannot be duplicated on successive runs even though the time of centrifugation is made as nearly the same as possible. Varying amounts of precipitated protein set up in duplicated lots and centrifuged for identical periods yielded results which were variable and which gave straight line curves with different slopes, none of




The volume of protein precipitated from a serum treated by the centrifuge method plotted against the percentages of protein shown in table 1.

which attained zero when projected. Therefore, when the protein content of an unknown serum is to be determined, it is necessary to use in the same experiment at least two different amounts of a serum with known protein content in order to obtain a curve characteristic of that run of the centrifuge. To this end a standard serum whose protein content was carefully established by the Kjeldahl method was distributed in 0.5 cc. amounts in small vials stoppered with paraffined corks and kept frozen. For each lot of determinations on unknown serums the content of one of these vials was thawed, and 0.2 and 0.1 cc. samples were carried through the procedure along with 0.2 cc. samples of the unknown serums. A straight line curve

was constructed from the results with the standard serum. Along this curve the volumes of precipitate from the unknown serums were located and the percentages of protein read.

For the determination of the globulin and the albumin of serum, the globulin is removed from the serum by half-saturation with ammonium sulfate, and the volume of the precipitate representing the remaining albumin is determined in the same manner as that of total protein. A sample of serum is well mixed with an equal amount of saturated solution of ammonium sulfate and centrifuged. The clear supernatant is pipeted off, and 0.4 cc. samples are used for the estimation of albumin. This amount contains the albumin remaining in 0.2 cc. of the serum after removal of the globulin in the manner described. From the volume of precipitate the percentage of protein as albumin is determined, the curve established with two amounts of standard serum being used as for estimation of total protein. It is necessary, of course, that this curve be constructed from readings made after the standard precipitates have been centrifuged along with the unknown albumin precipitate. Usually total protein and albumin are determined together. Subtracting the percentage of albumin from the percentage of total protein gives the percentage of globulin.

TABLE 2.—*Comparison of Results Obtained with the Centrifuge and the Kjeldahl Method*

| Centrifuge Method | | | Kjeldahl Method | | |
|---|---|---|---|---|---|
| Total Protein | Albumin | Globulin | Total Protein | Albumin | Globulin |
| 7.16 | 5.09 | 2.07 | 7.40 | 5.36 | 2.04 |
| 7.36 | 5.18 | 2.18 | 7.44 | 5.22 | 2.22 |
| 6.02 | 3.90 | 2.12 | 6.14 | 4.10 | 2.04 |
| 7.18 | 4.67 | 1.51 | 7.54 | 4.92 | 2.62 |
| 7.09 | 4.91 | 2.18 | 7.28 | 5.12 | 2.16 |
| 7.26 | 5.18 | 2.08 | 7.18 | 5.30 | 1.88 |
| 7.04 | 5.28 | 1.76 | 7.18 | 5.52 | 1.66 |
| 6.99 | 5.10 | 1.89 | 7.20 | 5.46 | 1.74 |
| 7.00 | 4.51 | 2.49 | 6.86 | 4.72 | 2.14 |
| 7.23 | 5.31 | 1.92 | 7.44 | 5.66 | 1.78 |
| 7.10 | 5.10 | 2.00 | 7.51 | 5.37 | 2.14 |
| 6.82 | 4.91 | 1.91 | 7.16 | 5.08 | 2.08 |
| 5.81 | 3.76 | 2.05 | 6.04 | 4.00 | 2.04 |
| 6.44 | 4.81 | 1.63 | 6.48 | 4.96 | 1.52 |
| 7.16 | 5.02 | 2.14 | 7.68 | 5.44 | 2.24 |

Removal of globulin with sodium sulfate according to the Howe method [8] was considered, but the use of ammonium sulfate was believed to be preferable. When protein is determined by its content of nitrogen the use of ammonium sulfate is interdicted. With the centrifuge method the nitrogen of ammonium sulfate is of no concern. When sodium sulfate is employed as described by Howe to remove globulins, the serum is diluted too much for convenient use with the centrifuge method. Moreover, the necessity of a period of three hours at 37 C. for precipitation of globulins is avoided when ammonium sulfate is used. Also the loss of albumin by adsorption on filtration, even when the best grade of filter paper is used,[9] and the error introduced by evaporation on filtration in a warm chamber are eliminated when ammonium sulfate is substituted for sodium sulfate.

8. Howe, P. E.: J. Biol. Chem. **49**:93, 1921.

9. Robinson, H. W.; Price, J. W., and Hogden, C. G.: J. Biol. Chem. **120**:481, 1937.

In table 2 are given the comparative results of a series of determinations of serum proteins made by the centrifuge method and by the Kjeldahl method, protein fractionation being made in the former with ammonium sulfate and in the latter with sodium sulfate as described by Howe. The greatest departure of the results for total protein with the centrifuge method from those with the Kjeldahl method was 5.8 per cent, the average being 3.2 per cent. For albumin the greatest departure was 7.0 per cent, with an average of 4.2 per cent.

The chief precautions to be taken in this method are: 1. Identical procedures must be used with each of the tubes in the same run of the centrifuge, including the speed and the manner of introducing the serum samples into the precipitant. 2. The measurements and the manipulations of each tube should be carried out as nearly together as possible. With observation of these two rules, duplicates are usually unnecessary. When total protein and albumin are determined on a single serum four places in the centrifuge are used. For the same determinations on each other serum run at the same time two more places are used. With a centrifuge having eight places total protein and albumin of three serums or total protein alone of six serums can be determined.

The following is a recapitulation of the method in outline form:

| | | | |
|---|---|---|---|
| Tube 1 | Measure into each 1.8 cc. of precipitant | Add 0.2 cc. standard serum | Mix contents of tubes thoroughly. Centrifuge 15 minutes at full speed. Read volumes of precipitates. Construct straight line curve with results from tubes 1 and 2. Locate readings of tubes 3 (total protein) and 4 (albumin) on curve. Read percentages of protein. Globulin equals total protein less albumin |
| Tube 2 | | Add 0.1 cc. standard serum | |
| Tube 3 (total protein) | | Add 0.2 cc. unknown serum | |
| Tube 4 (albumin) | | Add 0.4 cc. supernatant from centrifuged mixture of equal parts of unknown serum and saturated ammonium sulfate | |

Duplicate tubes 3 and 4 to the capacity of the centrifuge for determinations of the total protein and the albumin of additional serums. Omit tube 4 for determination of total protein alone and duplicate tube 3 with additional serums to the capacity of the centrifuge. Repeat tubes 1 and 2 with each run of the centrifuge.

This procedure provides a rapid and reliable method for the estimation of blood proteins. Except for the Bauer-Schenck centrifuge tubes, which are available commercially, no equipment is required other than that usually found in clinical laboratories.

SUMMARY

The method described for determining serum proteins depends on measuring the volumes of precipitated protein after the serum has been centrifuged in special graduated centrifuge tubes. Accuracy is obtained by the use of a standard serum to establish a curve of sedimentation characteristic of the conditions under which the determinations are made.

# Notes and News

**Army Institute of Pathology and American Registry of Pathology.**—What is now known as the Army Institute of Pathology was established in 1863 as the Army Medical Museum. During World War II the activities of the institute were greatly expanded, especially in the field of diagnostic pathology and research. There are now on file over 170,000 accessions. The results of research at the institute during the past few years will be published in a volume of about fourteen hundred pages as a part of the official history of World War II. The present director is Colonel J. Earl Ash, who will be succeeded on October 1 by Colonel Raymond O. Dart.

On request of Major General Norman T. Kirk, the Surgeon General of the Army, the Committee on Pathology of the National Research Council, Division of Medical Sciences, in late 1945 prepared a report on the future development of the institute. The report has been approved by the Surgeon General and by the War Department.

The essential recommendations in this report are (1) that a new building of adequate size be constructed; (2) that the Army Institute of Pathology be organized in four divisions—Department of Pathology, Army Medical Illustration Service, Army Medical Museum and American Registry of Pathology—each headed by a competent specialist; (3) that the staff of the institute be drawn from both the commissioned ranks of the Army and from the civilian professions; (4) that a comprehensive educational and training program be undertaken; (5) that the vast store of material at the institute be used for research and (6) that the services in pathology in the veterans' hospitals be centralized at the institute.

The American Registry of Pathology founded in 1922 thus is, and will continue to be, an integral part of the Army Institute of Pathology. There were, Jan. 1, 1946, over 43,000 cases registered. To effectuate the new plans as they relate to the registry, the National Research Council Division of Medical Sciences appointed a committee on the American Registry of Pathology. The members of the committee are Howard T. Karsner, chairman, Cleveland; Colonel J. E. Ash, Washington; Brigadier General George R. Callender, Washington; Colonel Balduin Lucké, Philadelphia; Robert A. Moore, St. Louis; Benjamin Rones, Washington; A. R. Shands Jr., Wilmington, Del., and Henry A. Swanson, Washington.

At the present time there are fourteen registries as a part of the American Registry of Pathology. These include Registry of Ophthalmic Pathology, established in 1922, sponsored by the American Academy of Ophthalmology and Otolaryngology; Lymphatic Tumor Registry, established in 1925, sponsored by the American Association of Pathologists and Bacteriologists; Bladder Tumor Registry, established in 1927, Kidney Tumor Registry, established in 1940, and Prostatic Tumor Registry, established in 1943, sponsored by the American Urological Association; Registry of Dental and Oral Pathology, established in 1933, sponsored by the American Dental Association; Registry of Otolaryngological Pathology, established in 1935, sponsored by the American Academy of Ophthalmology and Otolaryngology; General Tumor Registry, established in 1937, sponsored by the American Society of Clinical Pathologists; Registry of

Dermal Pathology, established in 1938, sponsored by the American Academy of Dermatology and Syphilology; Chest Tumor Registry, established in 1942, sponsored by the American Society of Thoracic Surgeons; Registry of Neuropathology, established in 1943, sponsored by the American Association of Neuropathologists; Registry of Orthopaedic Pathology, established in 1943, sponsored by the American Academy of Orthopaedic Surgeons; Registry of Veterinary Pathology, established in 1944, sponsored by the American Veterinary Medical Association, and Registry of Gerontology, established in 1945, sponsored by the Gerontological Society, Inc.

Plans for additional registries are under consideration. A professional scientific society wishing to sponsor a registry should communicate with the Director, Army Institute of Pathology, 7th Street and Independence Avenue, S.W., Washington 25, D. C. The society appoints a committee to work with the director in supervision of the activities of the registry, and makes an annual contribution to the budget, which is administered by the National Academy of Sciences.

All specimens in the registry are made available for review and research to competent investigators. Sets of slides and accompanying syllabuses on special field are available for loan to the civilian professions and officers of the federal services. Physicians, dentists and veterinarians are urged to send an unusual specimen together with an abstract of the history to the registry. The contributor receives a report on each specimen and is asked to keep the registry informed of the results of follow-up examinations of the patient.

With the reorganization of the Army Institute of Pathology to be completed during 1946 and 1947, a full time scientific director of the American Registry of Pathology will be appointed and sufficient clerks and technicians will be available to assure adequate use of the registries for diagnosis, research, training of young men and education of the professions.

# Books Received

EXPERIMENTAL HYPERTENSION. BEING THE RESULTS OF A CONFERENCE ON THIS SUBJECT HELD BY THE SECTION OF BIOLOGY OF THE NEW YORK ACADEMY OF SCIENCES, FEBRUARY 9 AND 10, 1945. Special Publications of the New York Academy of Sciences, volume 3, pages 1 to 180. Price $3.75. New York: New York Academy of Sciences, 1946.

This volume contains the papers read at the conference mentioned, with full reports of the discussions. The papers read are: "Introductory Lecture on the Production and Pathogenesis of Experimental Hypertension," by Harry Goldblatt. "The Mechanism of Renal Hypertension," by Eduardo Cruz Coke. "Rennin and Renal Hypertension," by L. F. Leloir. "The Problem of the Occurrence of Vasoconstrictor Substances in the Peripheral Blood of Hypertensive Patients and Dogs After Injection of Rennin," by Irvine H. Page, who also writes the preface. "Experimental Chronic Hypertension: Its Mechanism and Amelioration by Use of Various Blood Pressure–Reducing Substances," by Arthur Grollman. "Treatment and Prophylaxis of Experimental Renal Hypertension with Renal Extracts and Marine Oils," by G. E. Wakerlin and others. "Some Physiological Aspects of the Blood Pressure–Lowering Effect of Tissue Extracts in the Hypertensive Animal," by John W. Remington. "A Change of Mechanism in the Course of Hypertension of Renal Origin," by Eric Ogden and others. "Experimental Renal Hypertension and Amino Acid Metabolism in the Kidney," by Richard J. Bing.

A summary of the facts presented is made by William Goldring, who also writes the introduction and (with H. Chasis and H. W. Smith) submits a statement on the question of a similarity of pathogenesis of experimental renal hypertension and human hypertension.

The book reveals that experimental hypertensive research is in "a healthy state and one rich with promise." It is a highly important contribution.

AUTOPSY DIAGNOSIS AND TECHNIC. By Otto Saphir, M.D., pathologist, Michael Reese Hospital, and professor of pathology, University of Illinois Medical School, Chicago. Foreword by Ludvig Hektoen, M.D. Second edition, revised and enlarged. Pp. 405, with 69 illustrations. Price $5. New York and London: Paul B. Hoeber, Inc., 1946.

The revision has increased the usefulness of this valuable book. Diseases of the breast are now described with the care and the detail (11 pages) which their importance demands. Several new chapters have been added: autopsies on stillborn and other infants; anatomic findings in vitamin deficiencies; notes on the anatomic changes in certain tropical diseases; the nose and accessory sinuses. Certain chapters and tabulations have been enlarged—e. g., the chapter on unexpected death from natural causes, with notes on accidental death. The chapters on the autopsy permit, general technical considerations and the external examination are of special value.

PEPTIC ULCER: ITS DIAGNOSIS AND TREATMENT. By I. W. Held, M.D., attending physician, Beth Israel Hospital, and clinical professor of medicine (retired), New York University College of Medicine, New York; and A. Allen Goldbloom, M.D., assistant clinical professor of medicine, New York Medical College and Flower and Fifth Avenue Hospitals, associate physician, Beth Israel and Metropolitan hospitals, and associate cardiologist, Beth Israel Hospital, New York. Pp. 382, with 110 illustrations. Price $6.50. Springfield, Ill.: Charles C Thomas, Publisher, 1946.

ARTIFICIAL SUNLIGHT TREATMENT IN INDUSTRY: A REPORT ON THE RESULTS OF THREE TRIALS—IN AN OFFICE, A FACTORY AND A COAL MINE. By Dora Colebrook. Medical Research Council, Industrial Health Research Board, Report no. 89. Pp. 64. Price 30 cents. London: His Majesty's Stationery Office (New York: British Information Service), 1946.

ENVIRONMENTAL WARMTH AND ITS MEASUREMENT: A BOOK OF REFERENCE PREPARED FOR THE ROYAL NAVAL PERSONNEL RESEARCH COMMITTEE OF THE

MEDICAL RESEARCH COUNCIL. By T. Bedford. Medical Research Council War Memorandum no. 17. Pp. 40. Price, including charts for the calculation of environmental warmth, 2s 3d. London: His Majesty's Stationery Office, 1946.

ARQUIVOS DO INSTITUTO DE PATOLOGIA GERAL DA UNIVERSIDADE DE COIMBRA. Edited by Prof. Melico Silvestre and Prof. Mario Trincao. Volume 30. Pp. 488. Coimbra, Portugal, 1945.

The volume contains a report of 469 pages on new serologic aspects of typhoid by Henrique de Oliveira.

THE ROCKEFELLER FOUNDATION: ANNUAL REPORT, 1945. Pp. 346. New York: Rockefeller Foundation, 1946.

FORTY-THIRD ANNUAL REPORT, 1945-1946, IMPERIAL CANCER RESEARCH FUND, APRIL 1946. Pp. 41. London: Royal College of Surgeons, 1946.

THE PRINCIPLES AND PRACTICE OF TROPICAL MEDICINE. By L. Everard Napier, Companion of the Order of the Indian Empire; Fellow of the Royal College of Physicians of London; formerly director and professor of tropical medicine, Calcutta School of Tropical Medicine. Pp 917, with 195 illustrations. Price $11. New York: The Macmillan Company, 1946.

The objectives of this book are ". . . to give an accurate and concise account of the more important tropical diseases from the points of view of epidemiology, aetiology, pathology, symptomatology, diagnosis, prevention, treatment and prognosis, and to discuss in a general way such relevant subjects as methods for mitigating the effects of atropical climate, nutrition and anaemia in the tropics, and snakes and snake-bite." The book is planned for the student, the physician and the public health worker and is to be regarded rather as a textbook than as a book of reference. The author is well qualified by long experience, especially in India, to write authoritatively.

The book includes discussions of most of the tropical diseases and some that are not strictly tropical, such as tularemia, brucellosis and rabies. The author deliberately omits some diseases commonly found in the tropics, such as typhoid, tuberculosis, smallpox, most of the neurotropic viruses and the systemic mycoses, because they are described in textbooks of general medicine. He gives brief discussions of Rift Valley fever, melioidosis, parasprue, hill diarrhea, gnathostomiasis, lathyrism, infantile cirrhosis of the liver, anemia in the tropics, myiasis and some other topics not mentioned in all books on tropical diseases. The diseases with diarrhea are brought together, regardless of causes, into a section entitled "The Intestinal Fluxes"—an organization not commonly used, but useful nevertheless. The longest chapter (67 pages) is devoted to malaria.

This book is written from a practical point of view. The material is well organized, and the writing is clear and concise. The publisher has done an excellent job in printing, so that the headings and sections stand out distinctly. The historical, epidemiologic and geographic approaches are excellent. There are numerous good figures and 3 excellent plates in color, but photographs are not numerous and are of variable quality, probably because of the rough paper. Photomicrographs, for the same reason, are poor (e. g., figs. 167 and 168, and the photomicrographs on plate II), a point of importance to pathologists. The sections on pathology are generally briefer than most pathologists would like. Laboratory diagnostic procedures include those which the author has found useful. The bibliographies are short and are not intended to be exhaustive.

This book is up to date on most points, but it does not fully reflect the experiences of World War II with respect to tsutsugamushi, DDT as used in the control of mosquitoes and flies, the control of filariasis by taking advantage of the short flight range of infected mosquitoes, and the number of relapses of patients with Plasmodium vivax malaria from the Southwest Pacific. These criticisms are of minor importance. The book has so many excellent points that it stands out as one of the best books in one volume on tropical medicine. The author has admirably achieved his objectives, and the book can be highly recommended.